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SERUM GLUCURONIDASE ACTIVITY DURING NORMAL AND TOXEMIC PREGNANCY*

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THE introduction of a simple colorimetric method for the assay of mono-β-glucuronidase activity (22) has made the present study possible. Increased urinary excretion of estriol and pregnandiol glucuronide in pregnancy (4, 14, 23) has stimulated interest in serum glucuronidase activity in this condition. The present work establishes the serum glucuronidase level of normal and pregnant women and shows how the toxemias of pregnancy can be enzymatically differentiated.

During pregnancy many changes occur in the enzymes of the blood. These are reported as an increase in protease (1), histaminase (2, 15, 25) and amylase (16) activity; and a decrease in lipase (19). In addition, extracts of the posterior lobe of the pituitary gland are inactivated by pregnancy serum (24, 27) or upon injection into pregnant patients (6). This antagonism can be assayed as "pitocinase" according to Page (18).

During pregnancy toxemia a basic enzymatic disturbance beyond the normal changes seems to occur. There is a decrease in certain proteolytic enzymes (7, 8, 12, 17); although fibrinolytic activity increases (21, 26).

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Serum histaminase (2) and pitocinase (18) values are scattered on both sides of the normal curve, and there is no apparent correlation between their levels and the severity of the disease. The organism is more reactive than normal to injection of extracts of posterior pituitary, particularly to its pressor and antidiuretic components (6). A decrease in serum cholinesterase (13) may occur.

MATERIAL AND METHOD

A total of 190 women were studied. Except for a few designated cases, these were obtained from the surgical and obstetrical services of the Albert Merritt Billings Hospital and the Chicago Lying-In Hospital. Clinically two principle types of pregnancy toxemia are recognized; those peculiar to pregnancy, commonly referred to as pre-eclampsia, and those developing independent of the pregnant state, usually called hypertensive toxemia. The differentiation of these groups depends largely upon certain clinical findings, as age, symptoms, weight gain, degree and persistence of hypertension, edema and proteinuria. Such criteria are obviously only relative, not absolute. In this study the diagnosis of toxemia, and differentiation as to the type, was made according to accepted standards (7). Cases were considered to be either pre-eclamptic, eclamptic or hypertensive toxemia.

Mono-β-glucuronidase activity was determined by the method of Talalay, Fishman and Huggins (22), using 0.01 M. sodium-phenolphthalein glucuronide as substrate. The serum was obtained from clotted whole blood by centrifugation at high speed. Precautions to prevent hemolysis were taken, but this source of error was obviated by controls. Fresh tissues were thinly sliced, blotted to remove blood, and weighed. The weighed tissue was transferred to a chilled glass homogenizer, 5.0 cc. of distilled water were added, and the tissue was homogenized at high speed. After centrifugation, the supernatant was decanted and assayed in duplicate for glucuronidase activity. Results are expressed in micrograms of phenolphthalein liberated by 1.0 ml. of serum or by 1.0 Gm. wet weight of tissue in one hour under standard conditions of temperature and pH.

RESULTS AND INTERPRETATION

1. **Non-pregnant Controls:** In a group of 54 normal non-pregnant females varying in age from 2 to 60 years, the serum glucuronidase varied from 2.1 to 9.6 micrograms per ml. The mean and standard deviation for this group was 5.3 ± 2.1 micrograms per ml.

2. **Normal pregnancy:** Figure 1 illustrates graphically the mean curve and maximum variation for serum glucuronidase from one hundred normal pregnant women. Activity is plotted against the weeks gestation calculated from the last menstrual period. A downward inclination of the mean curve

at its inception is apparent between 6 to 12 weeks when serum gonadotropins likewise fall. The subsequent rise in serum glucuronidase parallels that of serum estrogens (20). Furthermore, the mean curve for glucuronidase levels off from 38 to 40 weeks, as does that of serum estrogen. It is significant that estrogen (and progesterone) are partly excreted as glucuronides. In a series of 9 normal post-partum women the serum glucuroni-

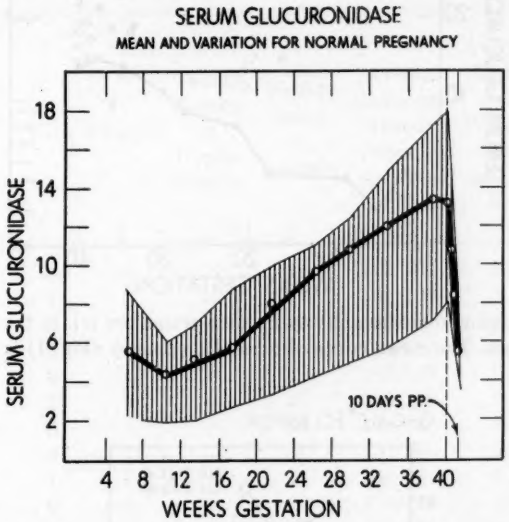

FIG. 1. Serum glucuronidase activity expressed in micrograms per ml. of one hundred normal pregnant women showing mean curve and variation.

dase level reached values less than 9.4 micrograms per ml. by the fifth to seventh post-partum day which is within the normal range for healthy controls.

3. **Toxemia of Pregnancy:** Figure 2 illustrates the serum glucuronidase activity in 24 cases of pregnancy toxemia as well as the relationship between these values and the normal mean curve. It becomes apparent that glucuronidase values in the 12 cases of hypertensive toxemia cluster about the mean glucuronidase curve, whereas those patients with pre-eclampsia (with two exceptions) have much higher values. In fact, a figure over 20 micrograms per ml. seems to indicate that the patient has pre-eclampsia. Of three patients with pre-eclampsia and one with eclampsia, three had reached normal values of serum glucuronidase by the eighth, eleventh and thirteenth post-partum days while one still had an elevated value upon discharge on the tenth day. Three normal patients who received 5 mg. of

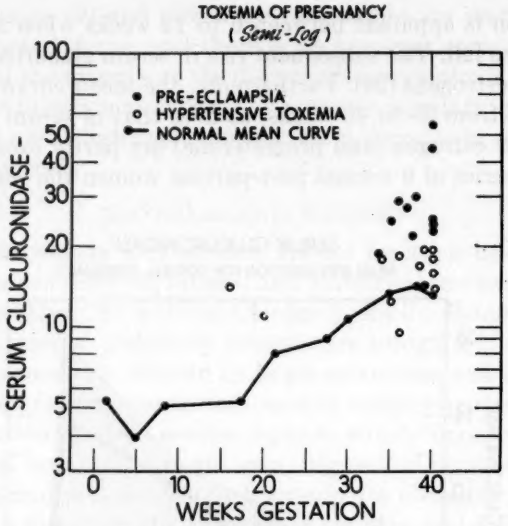

FIG. 2. Serum glucuronidase activity in micrograms per ml. in twenty-four cases of pregnancy toxemia. The mean curve of normal pregnancy (Fig. 1) is plotted for comparison.

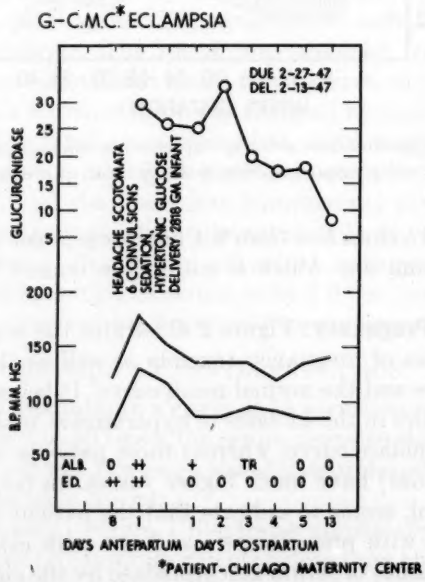

FIG. 3. Composite graph showing serum glucuronidase activity in micrograms per ml., systolic and diastolic blood pressure, albuminuria and edema during eclampsia.

diethyl stilbestrol daily during the first five post-partum days, to prevent lactation, continued to have elevated serum glucuronidase beyond the seventh post-partum day. The protocols for all patients with toxemia are given in Tables I and II. Figure 3 illustrates a composite graph for a patient with eclampsia showing the high glucuronidase levels in this syndrome with a decline to normal in thirteen days.

TABLE I. PRE-ECLAMPSIA

Identification	Age	Para	Gravida	Family History of Hypertension	Weeks Onset of Toxemia	Average Maximum Blood Pressure mm. Hg.	Average Maximum Proteinuria gm./24 hr.	Maximum Edema
339829	19	0	1	–	38	140/100	2.2	+
D.*	39	1	2	–	37	160/120	0.5	+
387436	25	0	1	+	40	186/90	9.1	+
387473	22	0	1	–	36	140/112	1.2	+
G.†	18	0	1	–	38	180/130	1.0	++
347493	23	0	1	–	37	156/100	1.3	++
383214	30	0	1	+	39	140/94	0.6	+
309066	35	6	7	–	34	155/90	11.1	++++
390760	22	1	2	–	38	140/90	0.2	++
389897	20	0	1	–	36	174/120	1.5	+++
392846	18	0	1	–	34	156/100	0.2	+++
182062	31	0	1	–	33	130/80	0.27	++

* Patient Lewis Memorial Hospital, Chicago, Illinois.
† Patient Chicago Maternity Center.

4. **Tissues:** The glucuronidase activity of 2 normal placentae was 1,772 and 2,185 micrograms per Gm. wet weight. The glucuronidase content of endometrium from a 7 and 40 week gestation was 580 and 360 micrograms per gram wet weight, respectively. Ovary and myometrium obtained at cesarean section of a 40 week gestation were 4,250 and 257 micrograms per gram. These values are low in comparison with liver, spleen and kidney from non-pregnant normal individuals, which were: kidney 6,850, liver 11,250 and spleen 6,500 micrograms per gram wet weight. This would indicate that the source of glucuronidase activity during pregnancy is not the gravid endometrium or the placenta.

5. **Infant and mother:** In a series of twelve comparative observations maternal and umbilical cord serum averaged 16.2 and 5.8 micrograms per

ml. respectively. In no case did the activity of cord blood equal or exceed that of the corresponding maternal blood. These observations exclude the fetus as a source of glucuronidase in the maternal serum; low values in the

TABLE II. HYPERTENSIVE TOXEMIA

Identification	Age	Para	Gravida	Family History of Hypertension	Weeks Onset of Toxemia	Average Maximum Blood Pressure mm. Hg.	Average Maximum Proteinuria gm./24 hr.	Maximum Edema
399793	42	1	3	+	16	176/110	0	+
386821	31	3	4	+	10	166/96	0	0
389267	25	2	3	+	20	170/90	0	++
389272	28	0	1	+	36	156/106	0	+++
C.*	25	0	1	+	20	155/90	0	0
389847	32	0	1	+	16	190/110	0	+++
394045	31	0	1	−	37	180/130	0	++
385358	27	0	1	+	39	164/102	0.4	0
307733	21	0	1	−	38	148/90	0	0
2242	37	3	2	+	36	184/104	0	0
D.R.†	25	2	3	+	32	154/100	0.2	+
387437	23	0	1	−	39	176/118	0.2	++

* Patient Roseland Community Hospital, Chicago, Illinois.
† Patient Lewis Memorial Hospital, Chicago, Illinois.

cord blood would indicate that the placenta was an effective barrier to the passage of the glucuronidase complex from maternal to fetal circulatory systems.

COMMENT

It is reported that the glucuronidase activity of the liver can be increased by administering borneol and menthol (10), and injection of estrogen increases the glucuronidase activity of the uterus (11). Moderately increased serum phenol levels during toxemia of pregnancy have been observed by Dieckmann (9). Glucuronidase probably regulates the conjugation of toxic substances having a free hydroxyl group with glucuronic acid (5) and thus increases their solubility and aids in their excretion by liver and kidney. However, the reason for the increased serum activity during pregnancy is not apparent. It would seem that the progressive increase of serum and urinary steroids (estrogens and pregnanediol)

during pregnancy would require a greater concentration of enzyme. On the other hand, the unusual increase of glucuronidase in the blood during pregnancy toxemia in the presence of a diminished serum and urine steroid content (22, 25) is puzzling, but may be compensatory. The evidence presented here lends support to those who suggest that pregnancy toxemia constitutes a basic enzymatic disturbance which is closely allied to estrogen, progesterone and gonadotropin metabolism. In addition study of serum glucuronidase seems to indicate that real differences between hypertensive toxemia and pre-eclampsia exist.

CONCLUSIONS

1. Progressive increase of serum glucuronidase activity occurs during normal pregnancy.
2. Serum glucuronidase is increased during pre-eclampsia and eclampsia, but is not significantly increased in hypertensive toxemia.
3. Determination of serum glucuronidase may be of value in the diagnosis of pre-eclampsia and eclampsia.
4. The source of the increased glucuronidase activity in the serum of pregnant women is unknown.

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RELATION OF URINARY STEROIDS TO THE DIAGNOSIS OF ADRENAL CORTICAL TUMORS AND ADRENAL CORTICAL HYPERPLASIA: QUANTITATIVE AND ISOLATION STUDIES

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CLINICAL pictures which accompany functioning tumors of the adrenal cortex are neither specific nor diagnostic. Other pathologic conditions produce similar or even identical symptoms. Only one of these other conditions need be mentioned here; namely, adrenal cortical hyperplasia.* For therapeutic reasons it is very important to distinguish cases of adrenal cortical tumor from adrenal cortical hyperplasia and from the other clinical and pathologic entities which simulate both of them. The discussion that follows centers around the chemical and biologic methods that can be employed to help make these distinctions.

Urine of patients who have functioning adrenal cortical tumors usually contains excessive amounts of both androgens and 17-ketosteroids. The former can be determined only by biologic methods, whereas the latter, which include not only androgenic steroids but others which seem to be biologically inert, can be determined by chemical methods. Kenyon, Gallagher, Peterson, Dorfman and Koch (1937) (27) were among the first to demonstrate that there may be large amounts of androgenic material in the urine excreted by patients having these tumors. They studied fifteen cases of "virilism" among women. Twelve patients excreted normal amounts of androgens, two excreted 25 per cent more than normal and one, who had a carcinoma of the adrenal cortex, excreted very large amounts. Similar observations were made by R. K. Callow (1938) (11). He determined both the androgen and the 17-ketosteroid content of the urine. In his series of cases there was one woman who had Cushing's syndrome, also a girl aged nineteen years who had an adrenal cortical tumor,

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* Detailed descriptions of the clinical symptoms which accompany adrenal cortical tumors and hyperplasia may be found in reviews by Haymaker and Anderson (1938) (25), by Kepler and Keating (1941) (28), by Cahill (1944) (7), by del Castillo (1941) (12) and by Kenyon (1944) (26).

The term "hyperplasia" is used here in a loose clinicopathologic sense to denote bilaterally enlarged hyperfunctioning adrenal cortices associated with such clinical pictures as pseudohermaphroditism, adrenal virilism, Cushing's syndrome and others which also may occur in conjunction with adrenal cortical tumor.

and several women who were virilized but who presumably did not have adrenal cortical tumor. Only in the case of adrenal tumor was there found an increase of the urinary content of both androgenic and 17-ketosteroidal material. Callow therefore came to the conclusion that "adrenal tumor can be diagnosed with the aid of androgen assay." Levy Simpson (1938) (31), however, in a discussion of Callow's paper, pointed out that Callow's conclusion should be qualified since, in Levy Simpson's experience, the urine of patients having adrenal cortical hyperplasia sometimes contained amounts of androgenic material comparable to those obtained in cases of adrenal cortical tumor.

Shortly before these developments Frank (1934, 1937) (18, 19) made the astounding and seemingly paradoxical discovery that the estrogen content of the urine was increased in four cases of adrenal cortical tumor. From these observations he concluded that in the absence of pregnancy the presence of 500 to 1,000 mouse units of estrogenic substance per liter of urine should be considered strong evidence in favor of adrenal carcinoma. Frank's observation was confirmed in isolated instances in which the patients were women reported by Graef, Bunim and Rottino (1936) (24), by Lukens and Palmer (1940) (33), by Cahill, Loeb, Kurzrok, Stout and Smith (1936) (8) and by McGavack (1940) (34) and in a case in which the patient was a feminized man reported by Levy Simpson and Joll (1938) (32). However, it soon became apparent from the case reports of Walters and Kepler (1938) (48), of Dorfman, Wilson and Peters (1940) (15) of Crooke and R. K. Callow (1939) (13) and of Bruins Slot (1936) (4) that a functioning adrenal cortical tumor of women usually was not associated with the urinary excretion of large amounts of estrogenic material.

Knowing that the urine of patients having adrenal cortical tumors contained androgens, investigators naturally tried to isolate the specific compounds which might be responsible for the androgenic activity. Some of the early work along this line was done by Crooke and R. K. Callow (1939) (13). Four cases of Cushing's syndrome were investigated. In two of these an adrenal cortical tumor was responsible for the clinical picture. In the other two cases, in which adrenal cortical tumor was excluded, the excretion of both androgens and 17-ketosteroids was found to be relatively normal. In the former it was shown that the excretion of both of these groups of substances was increased and that a specific compound, dehydroisoandrosterone, was largely responsible for the increased excretion of 17-ketosteroids. Subsequently, these observations were confirmed. Talbot, Butler and MacLachlan (1940) (43) Fraser, Forbes, Albright, Sulkowitch and Reifenstein (1941) (20) Patterson, McPhee and Greenwood (1942) (40) and Friedgood and Whidden (1939) (22) verified the increased excretion of 17-ketosteroids and Wolfe, Fieser and Friedgood (1941) (50)

Warren (1945) (49) and N. H. Callow and Crooke[1] (1944)(9) verified the observation that the excretion of dehydroisoandrosterone was increased.

As experience with the determination of the urinary 17-ketosteroids increased it soon became evident that increased values were not pathognomonic for adrenal cortical tumor. To increase the specificity of the test, Talbot, Butler and MacLachlan (1940) (43) devised a method for the determination of the 3(β)-alcoholic fraction of these substances. This fraction may be made up of several 3(β)-alcoholic steroids, among which is the compound previously mentioned, dehydroisoandrosterone. They found that the 3(β)-alcoholic fraction formed not more than 10 per cent of the 17-ketosteroids for normal men, women and children, while it accounted for 50 and 63 per cent of the total in two cases of adrenal tumor. In a later study of four cases of adrenal tumor, Talbot, Butler and Berman (1942) (42) found 3(β)-alcoholic fractions of 22, 49, 65 and 38 per cent of the total 17-ketosteroids. In Friedgood's (1944) (21) experience cortical hyperplasia was associated with a 3(β)-alcoholic fraction of less than 25 per cent and cortical tumors (three cases) were associated with 3(β)-alcoholic fractions of 30 to 70 per cent of the total 17-ketosteroids.

On the basis of work done by Butler and Marrian (1937, 1938) (5, 6), Broster and Vines (1937) (3) suggested that the presence of the compound pregnane-3(α),17,20-triol in urine was a phenomenon specific for virilism of adrenal origin. Butler and Marrian felt that the suggestion of Broster and Vines was justified since the compound had been found only in the urine of patients having adrenal virilism (two cases of adrenal hyperplasia).

Recently, Anderson, Hain and Patterson (1943) (2) described a case of adrenal tumor which was associated with an abnormally high excretion of pregnanediol (12 to 20 mg. per day) as well as large amounts of 17-ketosteroids (215 mg. on one day). Pregnane-3(α),17,20-triol was not found. They inferred that a diagnosis of adrenal tumor or of adrenal hyperplasia could be made on the basis of the pregnanediol output alone. Venning, Weil and Browne (1939) (47) and Salmon, Geist and Salmon (1941) (41) also isolated sodium pregnanediol glucuronidate from the urine of patients who had adrenal carcinoma and of patients who had adrenal hyperplasia. Furthermore, Genitis and Bronstein (1942) (23) reported data on two female pseudohermaphrodites, four and ten years old, who excreted as much as 20 mg. of pregnanediol in forty-eight hours. The excretions of 17-ketosteroids were 27 mg. and 30 to 69 mg. in twenty-four hours. However, Talbot, Butler and Berman (1942) (42) found that the pregnanediol values were not consistently elevated in patients having adrenal cortical hyperplasia.

[1] Callow and Crooke (1944) (9) reported one case of adrenal cortical tumor in which the excretion of 17-ketosteroids was not increased.

In brief, the literature shows clearly that the determination of the urinary estrogens, androgens and 17-ketosteroids is a valuable tool in the recognition of adrenal cortical hyperplasia and functioning adrenal cortical tumor and that these two conditions frequently can be distinguished one from another by determining the proportion of the 3(β)-alcoholic 17-ketosteroids. It is also clear that pregnanediol may be excreted in the urine by patients having either adrenal cortical tumor or adrenal cortical hyperplasia and that pregnane-3(α),17,20-triol may be present in the urine of patients suffering from adrenal virilism associated with adrenal cortical hyperplasia. On the other hand, it is evident that excessive amounts of urinary androgens, estrogens and 17-ketosteroids are not invariably present in the urine in cases of adrenal cortical tumor, that the differential diagnosis of adrenal cortical tumor and adrenal cortical hyperplasia cannot always be made by determining the androgenic content of the urine and that adrenal cortical hyperplasia is not necessarily characterized by the presence of pregnanediol in the urine. Finally, it is evident that investigators have been handicapped by having only small series of cases and sometimes have been inclined to infer too much from too little.

The report that follows deals with data that we have collected from patients having either adrenal cortical tumors or adrenal cortical hyperplasia.

MATERIAL AND METHODS

Details of the clinical records will be submitted for publication in a separate report. A summary of the significant abnormalties is presented in table 1. In tabulating the cases we attempted to follow the classification recently proposed by Kenyon (1944) (26). His proposals were modified slightly so as to include not only the neoplasms but also the so-called hyperplastic lesions:

Clinical pictures associated with hyperfunctioning adrenal cortical lesions.

1. Adrenogenital syndrome (including pseudohermaphroditism).
2. Cushing's syndrome.
3. Intergrades between types 1 and 2.
4. Isolated expressions of the lesion.
5. Feminization.
6. Lesions without endocrine manifestations.

For various reasons the practical application of this classification, or for that matter any other, is often very difficult when one is dealing with specific cases. We have discussed this problem in a recent paper which was

TABLE 1. CLINICAL OBSERVATIONS

Case	Age, years	Sex	Pathologic lesion*	Blood pressure, mm. of mercury	Diabetes, requiring insulin	Osteoporosis	Weakness	Striae	Hirsutism	Acne	Enlarged clitoris	Florid color	Obesity	Glucose tolerance curve†	Remarks
3	63	F	T	162/88		+	+	−	+	−	−	+	−	92, 194, 208, 102	Predominantly Cushing's syndrome
5	25	F	T	204/115		+	+	+	+	−	−	+	−	77, 112, 154, 104	Predominantly Cushing's syndrome
6	3	F	T	110/80		−	−	−	−	+	+	+	−		Adrenogenital syndrome with somatic and heterologous sexual precocity
7	27	F	T	118/68		−	+	−	+	−	−	−	−	71, 109, 176, 78	Isolated endocrine symptoms
8	45	F	T	225		−	−	−	+	−	+	+	−	85, 118, 208, 149	Adrenogenital syndrome
9	3	F	H	110/68		−	−	−	−	−	+	−	−		Feminine pseudohermaphrodite with pubertas praecox
10	5	F	H	100/60		−	−	−	−	−	+	−	−		Feminine pseudohermaphrodite with pubertas praecox
11	19	F	H	110/80		−	−	−	+	−	+	−	−		Feminine pseudohermaphrodite
12	25	F	T	160/140		−	+	+	+	−	+	−	−	"Diabetic" type	Predominantly Cushing's syndrome with features of the adrenogenital syndrome
13	9	M	H	120/70		−	−	−	−	−		−	−		Homclogous pubertas praecox Adrenogenital syndrome (?)
14	53	F	T	160/84		+	+	−	+	+	+	+	−		Predominantly Cushing's syndrome
15	55	F	T	100/60					+				−		Incompletelystudied; metastasis present
16	40	F	T	154/92		−	−	+	+	+	+	+	+		Predominantly Cushing's syndrome
17	39	M	T	145/90	+‡	−	−	−		−		−	−		Nonfunctioning (?) neoplasm
18	32	M	T	110/72			−	−		−		−	−	86, 107, 90, 83	No clinical endocrine abnormalities but increased excretion of 17-ketosteroids
19	10	F	H	120/80		−	−	−	−	−	+	−	−		Feminine pseudohermaphrodite
20	27	F	H	160/76	+	−	+	−	−	+	−	+	+		Cushing's syndrome
21	29	M	H	175/135		+	−	−		−		+	+		Cushing's syndrome
22	26	F	H	150/105		−	+	+	+	−	−	+	−		Cushing's syndrome

* H = hyperplasia; T = tumor.

† Blood glucose in mg. per 100 c.c. The values were obtained, in the order given, before and ½ hour, 2 hours and 3 hours after administration of glucose.

‡ Diabetes was present after removal of the tumor but in milder degree than before removal.

presented at the 1946 Laurentian Hormone Conference (Kepler, et al. 1946) (29).

The diagnosis in all but two instances was established by surgical ex-

ploration. These two cases deserve brief comment. One patient (case 12), aged twenty-five years, had acquired symptoms of an adrenal cortical tumor at the age of eighteen years. Her habitus was not unlike that of an achondroplastic dwarf. In addition, she had many of the features of Cushing's syndrome but the features of virilism were more marked than those usually encountered in this clinical picture. In the upper right quadrant of the abdomen there was a large tumor which had its origin above the right kidney and there were shadows in the roentgenograms of the lungs which had the characteristics of metastatic malignant lesions. Under the circumstances there seemed to be no point in establishing the diagnosis surgically. One can be reasonably certain, however, that the patient did have an adrenal cortical tumor but the diagnosis cannot be regarded as proved. The other patient was a boy with homologous sexual precocity. His sister, the patient in case 9, had a similar disorder but the sexual precocity was heterologous in type. Her adrenal glands were explored surgically and found to be hyperplastic. It is likely, therefore, that the adrenal glands in the boy's case were hyperplastic, although here again the diagnosis cannot be regarded as proved.

The specimens of urine for quantitative determinations were collected in brown bottles containing 5 ml. of concentrated hydrochloric acid. The fresh specimens were boiled for ten minutes after addition of 0.1 volume of concentrated hydrochloric acid and then were extracted with carbon tetrachloride. The extracts were partially purified by the procedure of Talbot, Butler, MacLachlan and Jones (1940) (45). The method of N. H. Callow and associates (1938) (10) was used for determination of the total 17-ketosteroids with application of a correction equation to compensate for overestimation due to nonketonic chromogens (Engstrom and Mason, 1943 (16)). A modification of the method of Talbot, Butler and MacLachlan (1940) (43) was used for the determination of the 3(β)-alcoholic 17-ketosteroid fraction.

The procedures used for isolation of the various steroids have been described elsewhere (Mason and Kepler, 1945) (37).

RESULTS

With Engstrom (1944) (17) in a previous paper we have presented quantitative data on the urinary excretion of 17-ketosteroids in eight cases of proved adrenal cortical tumors and three cases of cortical hyperplasia. Eight of these cases are included in the present studies and have been assigned the same numbers used in the previous report. In table 2 are presented further quantitative data on cases 5 and 11, together with data obtained in five additional cases of tumor and four of hyperplasia. The condition of the adrenal glands was established by surgical exploration in

all of the cases presented in table 2. The patients in cases 11 and 19 were pseudohermaphrodites. The patients in cases 20, 21, and 22 had frank, easily recognized Cushing's syndrome. In the light of the diagnostic criteria proposed by Crooke and R. K. Callow (1939) (13) and by Talbot and his associates (1940) (44) and mentioned previously in this report, cases 16 and 17 are particularly interesting. One of these patients (case

TABLE 2. THE 17-KETOSTEROID EXCRETION OF PATIENTS WHO HAD ADRENAL CORTICAL TUMORS OR HYPERPLASIA

Case	Age, years	Sex	Specimens collected	Average 17-ketosteroid excretion per 24 hrs., mg.	β-Fraction, average per cent of total 17-ketosteroids
Adrenal cortical tumor					
5	26	F	3	107*	60
14	53	F	1	800	69
15	55	F	1	1,005	77
16	40	F	2	30	7.5
17	39	M	2	4.8	—
18	32	M	2	68	48
Adrenal cortical hyperplasia†					
11	21	F	1	123‡	20
19	10	F	2	31	14
20	27	F	3	24	14
21	29	M	2	28	2
22	26	F	2	10	5

* A preoperative value of 130 mg. for this case was reported previously. The above value was obtained after recurrence of the tumor approximately one year later.

† In cases 11 and 19 the patients were female pseudohermaphrodites with masculine characteristics. In cases 20, 21 and 22 the patients had Cushing's syndrome.

‡ A value of 75.2 mg. was reported previously. The above value was obtained approximately two years later.

16) had a functioning adrenal cortical tumor. The value for the total excretion of 17-ketosteroids per twenty-four hours (30 mg.), although well above the normal range, was not greatly elevated and was within the range found in one previous case and in three of the present cases of hyperplasia. The 3(β)-alcoholic fraction of the 17-ketosteroids was well within normal limits. In case 17 the tumor probably was not functioning. The value of 4.8 mg. for the total 17-ketosteroids was definitely less than nor-

mal. Our lowest normal value for a man is 6.0 mg. Because of the low value the 3(β)-alcoholic fraction was not determined in case 17.

There is already evidence in the literature that occasionally an adrenal cortical tumor may be present without a significant increase of the urinary excretion of androgens or 17-ketosteroids. Lawrence (1937) (30) described a case in which the excretion of androgens was only 1 to 2 capon units in twenty-four hours. Albright[2] (1943) (1) referred to two cases with proved tumors in which the excretion of 17-ketosteroids was 13.7 and 10.2 to 11.1 mg. in twenty-four hours and N. H. Callow and Crooke (1944) (9) reported one case of a woman sixty-one years of age who excreted 14.5 to 20 mg. per day.

In the five cases of hyperplasia one value for the 17-ketosteroids was normal, three were moderately elevated and one was greatly increased. The present value for case 11 represents an increase of 64 per cent in the amount excreted as compared to the value obtained two years previously. The 3(β)-alcoholic fraction of the 17-ketosteroids of the group of cases with hyperplasia comprised 2 to 20 per cent of the total.

Table 2 reveals that, with two exceptions, a consideration of both the total and the 3(β)-alcoholic fraction of the 17-ketosteroids distinguishes cortical tumors from hyperplasia. Case 16 could not be differentiated from hyperplasia on this basis. Determination of the 17-ketosteroids was sufficiently high to suggest the presence of a tumor but the 3(β)-alcoholic fraction was only moderately increased.

Table 3 summarizes the results of the isolation of steroids from the urine in six cases of cortical tumor and four of hyperplasia. A detailed account of the chemical procedures and substances isolated has been given elsewhere (Mason and Kepler, 1945) (37). Because of wide variations in the amounts of urine available and changes in the procedures used, the quantities given are of doubtful significance for comparison of the steroid excretion in one case with that in another. However, when possible, the values have been calculated in terms of the amount per day and included in the table in parentheses.

Deydroisoandrosterone was obtained in large amounts from all but one of the cases of tumor. In each instance the amounts isolated were greater than the sum of the amounts of the other ketones isolated. This result further substantiates the suggestion of Crooke and R. K. Callow (1939) (13) that cortical tumors are usually accompanied by the excretion of excessive amounts of this substance. On the other hand the amount of

[2] Recently Albright (personal communication) has had two additional cases in which the values were normal, and we also have recently had a case of this type.

dehydroisoandrosterone that could be isolated in the cases of hyperplasia was a small fraction of the ketones isolated. In one case none could be found. Case 12 was exceptional among the cases of tumor in that no dehydroisoandrosterone could be isolated. A 17-ketosteroid determination was not made but a bio-assay for androgens indicated the presence of the equivalent of not more than 5 mg. of androsterone in the urine excreted during a twenty-four hour period.

TABLE 3. SUMMARY OF THE STEROID FRACTIONS ISOLATED FROM THE URINE OF PATIENTS WITH ADRENAL CORTICAL TUMORS OR HYPERPLASIA

		Urine		Substances isolated											
				Neutral extract			Ketones					Nonketones		
Case	Age, years	Volume, liters	Days	Total wt., mg.	Ketonic fraction, mg.	Nonketonic fraction, mg.	Dehydroisoandrosterone, mg.	Androsterone, mg.	Etiocholan-3(α)-ol-17-one, mg.	Androstane-3(α), 11-diol-17-one, mg.	Estrone, mg.	Pregnane-3(α), 20(α)-diol, mg.	Pregnane-3(α), 17, 20-triol, mg.	Pregnane-3(β), 20(α)-diol, mg.
Adrenal cortical tumors														
3	63		14	1,450 (104)*	570 (39.3)	6,400 (457)	184 (13.1)		65 (4.6)	30 (2.1)		28 (2.0)		
5	25†	43.0	26	9,300 (358)	5,600 (216)	2,900 (112)	2,190 (84.3)	49 (1.9)	192 (7.4)	11 (0.4)	62 (2.4)	107 (4.1)		
6	3	6.4	13	1,430 (110)	790 (60.8)	710 (54.6)	1,525 (117.3)		33 (2.5)			53 (4.1)		
7	27	5.7	3	1,570 (523)	870 (290)		391 (130.3)		13 (4.3)			13 (4.3)		
8	45	50.4	31	8,000 (258)	3,500 (113)	3,000 (96.8)	740 (23.8)	290 (9.3)	127 (4.1)	77 (2.5)		90 (2.9)		
12	25	15.0	13	3,200 (246)	1,250 (96.2)	1,630 (125.3)		55 (4.2)				127 (9.8)	47 (3.6)	
Adrenal cortical hyperplasia														
9	3	18.0		1,200	320	590	33‡	55‡		65‡			27	
13	9	18.0		1,160	520	470						Trace	35	12
10	5	11.0	13	1,350 (104)	410 (31.5)	410 (31.5)		19 (1.5)	11 (0.8)	23 (1.8)		71 (5.5)		
11	19†	54.0		4,480	2,010	1,650	7	122		80		219	74	6

* The values in parentheses are those calculated as the amounts obtained per day.

† The data given in this table were obtained before the data in table 2, hence the difference in the ages in cases 5 and 11 in the two tables.

‡ The ketonic fractions in cases 9 and 13 were combined by mistake and therefore were worked up together.

Androsterone was found consistently in the urine in the cases of hyperplasia but in only three of the cases of tumor. Etiocholan-3(α)-ol-17-one was isolated in all of the cases of tumor in which dehydroisoandrosterone was found but in only one case of hyperplasia. The significance of these variations is not apparent but the results are in general agreement with the quantitative results of Dobriner and his associates (1942) (14). In their one case of adrenal tumor the urine contained androsterone while in two of their cases of hyperplasia very little etiocholan-3(α)-ol-17-one could be found. Recent studies (Mason and Kepler, 1945, 1947) (36, 38) of the metabolism of dehydroisoandrosterone suggest that the variations may be due to individual differences in the conversion of this substance to androsterone and etiocholan-3(α)-ol-17-one.

Androstane-3(α),11-diol-17-one (11-hydroxyandrosterone) is a recently discovered compound (Mason and Kepler, 1945). It has been found recently (Mason, 1946) (35) also in normal human male urine to the extent of 0.3 mg. per liter. It was found to be present in considerably increased amounts in most of these cases of adrenal cortical disease. Miller, Dorfman and Sevringhaus (1946) (39) have recently isolated very large amounts (10 mg. per liter) of this substance from the urine of a woman who had an adrenal tumor. Presumably it is a metabolic product of some of the adrenal steroids which have a hydroxyl group at carbon number eleven. Corticosterone, 17-hydroxycorticosterone and related hormones suggest themselves as likely precursors. If so, an increased production of these cortical hormones by the tumors and hyperplastic cortices is indicated.

Although Broster and Vines (1937) (3) and Butler and Marrian (1938) (6) have emphasized the relation of pregnane-3(α),17,20-triol to adrenal cortical hyperplasia it is reasonable to suspect that it would also occur in the urine of patients who have cortical tumors. It was found in three cases of hyperplasia and one case of tumor. Elution of the chromatogram in case 6 was not carried far enough to obtain this substance if present. It definitely was not found in the urine in cases 3, 5, 7 and 8 and previous experience makes it fairly certain that had it been present in appreciable amounts it would have been found. It is not known how easily this pregnanetriol is destroyed by the hydrolysis of the urine but preliminary experiments with a mixture of sodium pregnanediol and pregnanetriol glucuronidates which was isolated from the urine in case 11 indicate that pregnane-3(α), 17, 20-triol is more sensitive to the hydrolytic procedure than pregnanediol.

The inference of Anderson, Hain and Patterson (1943) (2) that a diagnosis of adrenal tumor or of adrenal hyperplasia could be made on the basis of the output of pregnanediol receives support from our results except for

the brother and sister who had hyperplasia (cases 9 and 13). There was, however, in the urine of the boy (case 13) an isomer of the usual pregnanediol (pregnane-3(α),20(α)-diol). The isomer had the properties of pregnane-3(β),20(α)-diol. It was also present in the urine in case 11.

Although pregnane-3(α),20(α)-diol was isolated after hydrolysis it could not always be obtained as sodium pregnanediol glucuronidate by the usual procedure of Venning (1937) (46). This procedure, with some modifications required by the large amount of material in the butyl alcohol extract, was used for cases 3, 5, 6 and 8 of adrenal tumors and for case 11 of hyperplasia. Sodium pregnanediol glucuronidate was isolated in cases 3, 6 and 11. The amount in case 6 was only a trace of crystals which after two recrystallizations had the correct appearance but the rather low melting point of 252–253°. Venning gave a melting point of 268–271° with decomposition. The glucuronidate obtained from case 3 corresponded to 5 and 13 mg. of pregnanediol in two determinations. The pregnanediol glucuronidate obtained in case 11 was accompanied by a glucuronidate which gave pregnane-3(α),17,20-triol on hydrolysis. It was not possible to separate the two glucuronidates by recrystallization. A crystalline precipitate which was obtained in case 8 when sodium pregnanediol glucuronidate was sought was identified as sodium dehydroisoandrosterone sulfate, which was later obtained in large amounts from this urine. A crystalline precipitate could not be obtained at the proper point from the urine in case 5 even though a relatively large amount of pregnanediol was isolated after hydrolysis of the urine.

Although, as has been mentioned elsewhere, Frank (1934, 1937) (18, 19) described two consecutive cases and later two more of adrenal tumor with greatly elevated excretions of estrogens, in only one of our cases was an abnormal amount excreted. One assay of the urine in case 5 gave a value of 9,000 rat units in twenty-four hours. Assay of the extract of the pooled urine gave an average value of 3,400 rat units for twenty-four hours or a total for twenty-six days of 88,400 rat units, which is equivalent approximately to 177 mg. of estrone. Actually, 62 mg. of estrone (m. p. 247–253°) were isolated. Recrystallization raised the melting point to 256°. The melting point was not depressed by admixture with authentic estrone. Although crystals were obtained from the fractions which may have contained estradiol and estriol, the melting point of the crystals did not correspond to that of either of these substances.

COMMENT

Consideration of the data presented shows that there is no single chemical criterion by which an adrenal cortical tumor can always be differen-

tiated from cortical hyperplasia. The most valuable contributions of the laboratory are determinations of the total 17-ketosteroids and of the 3(β)-alcoholic fraction. These two determinations usually help to distinguish between a tumor and hyperplasia. However, it is possible for a functioning adrenal tumor (case 16) to be associated with a value for the 17-ketosteroids that is only two to three times the average normal value and with a 3(β)-alcoholic fraction that is not increased beyond the normal range of values. Such a combination of results would ordinarily be interpreted as indicative of cortical hyperplasia.

Assay of the urine for estrogens may occasionally indicate the presence of a tumor. No cases have been reported in which excessive excretion of estrogens was associated with cortical hyperplasia.

Very little attention has been paid to the nonketonic fraction. A determination of pregnanediol or of 17,20-dihydroxy compounds such as pregnane-3(α),17,20-triol may well be informative in some instances. Unfortunately our results indicate that, although pregnanetriol is found more often in association with hyperplasia, it may occasionally be found in association with a tumor. Increased amounts of pregnanediol may be found in cases of tumor and of hyperplasia. In this group of cases it was present more consistently in the urine in cases of tumor than in cases of hyperplasia.

SUMMARY

The urinary 17-ketosteroids and the 3(β)-alcoholic fraction of the 17-ketosteroids were determined in six cases of adrenal cortical tumor and five cases of cortical hyperplasia. The ketonic and nonketonic steroids were isolated from the urine in six cases of tumor and four of hyperplasia.

In one case of tumor the quantitative determination of 17-ketosteroids gave a result below the normal range of values. In another case, the 3(β)-alcoholic fraction was only 7.5 per cent of the total 17-ketosteroids (30 mg.). In the four other cases of tumor the 3(β)-alcoholic fraction was 48 to 77 per cent of the total amounts (68 to 1,005 mg.) of 17-ketosteroids. In cases of hyperplasia the 3(β)-alcoholic fraction was 2 to 20 per cent of the total 17-ketosteroids (10 to 123 mg.). Consideration of these results in conjunction with those of other investigators indicates that excretion of 50 mg. or more of 17-ketosteroids per day with a 3(β)-alcoholic fraction of 50 per cent or more is strong evidence in favor of the presence of adrenal tumor. In a few instances, however, an adrenal tumor may be present when these values are considerably lower and within the range of values which have been found in association with cortical hyperplasia.

The results of the isolation studies emphasize again the relatively large

amounts of dehydroisoandrosterone which are excreted in cases of adrenal tumor. Very little of this substance was isolated in the cases of hyperplasia. Androsterone and etiocholan-3(α)-ol-17-one were isolated, the latter more consistently in the cases of tumor, the former in all cases of hyperplasia. Pregnane-3(α),20(α)-diol was isolated in all cases of tumor and in three cases of hyperplasia. Pregnane-3(β),20(α)-diol was tentatively identified in two cases of hyperplasia. Pregnane-3(α),17,20-triol was found in one case of tumor and in three cases of hyperplasia. A new 17-ketosteroid, androstane-3(α),11-diol-17-one, was isolated in three cases of tumor and in all of the cases of hyperplasia. It appears to be related to the 11-oxygenated adrenal hormones. Excessive excretion of estrogens was observed in only one case (of tumor) and estrone was isolated from the urine in this case.

Pregnane-3(α),17,20-triol appears to be found more often in association with cortical hyperplasia than with tumors. Examination of the urine for this substance may well be of value for confirmation of a diagnosis of cortical hyperplasia.

Finally, our observations, taken in conjunction with others reported in the literature, indicate that every patient presenting symptoms suggesting adrenal cortical tumor or adrenal cortical hyperplasia must be studied individually, using both clinical and laboratory procedures. Neither is self-sufficient.

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PLASMA PROTEIN PATTERN (TISELIUS ELECTROPHORETIC TECHNIQUE) IN CUSHING'S SYNDROME

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DOUGHERTY and White (7) reported an increase of the serum γ-globulin in rabbits following injection of large amounts of adrenal extract, and a release of γ-globulin antibody from lymphoid tissue following adrenal stimulation by adrenotrophic hormone, or following injection of adrenal extract (2). These observations suggested the question of what changes, if any, occur in the γ-globulin level of the plasma of patients with hyperadrenal function of the Cushing's syndrome type.

METHODS

A group of 9 patients proved by physical examination and pertinent laboratory findings to have Cushing's syndrome were studied. The results were contrasted with those obtained on patients with Addison's disease (5) and hypo-adrenal function of pituitary origin (6). In 8 of the patients with Cushing's syndrome, the plasma proteins were studied before treatment was instituted. Three of this group were observed for a period of as long as three years after therapy. One patient, no. 9, who had had a partial adrenalectomy and x-ray therapy to the pituitary gland, was studied five and a half years after treatment. He was in excellent condition at the time of the study. The plasma protein patterns of 4 patients with acromegaly were also studied.

The Tiselius electrophoretic technique as modified by Longsworth was employed. The method of calculating the results and the normal values obtained in this laboratory have been given in detail (3).

RESULTS

The data obtained on the 8 patients who showed evidence of adrenal cortical hyperactivity as judged by the presence of clinical signs of Cushing's syndrome, is summarized in Fig. 1. In all cases the plasma γ-globulin level was decreased below normal. The α_2-globulin was above the highest normal level in 3 cases and above the average normal value in all cases, and the albumin was decreased in 7. There was no significant change in the total protein level, the β-globulin or fibrinogen.

Three of the eight patients, and one (no. 9) on whom no pretreatment picture was obtained were given x-ray irradiation to the pituitary gland.

Hemiadrenalectomy of both adrenal glands was also carried out on these patients. The plasma protein picture (no. 2, 3, and 6) did not return to normal, although there was some increase in the γ-globulin level in no. 2 and no. 6 (Table 1). The plasma protein pattern of case 9 varied only slightly from normal. It is interesting that the clinical condition of this patient was also essentially normal at the time the study was made.

PLASMA PROTEIN PATTERN OF PATIENTS WITH MARKED HYPERADRENAL CORTICAL ACTIVITY, CUSHING'S SYNDROME TYPE

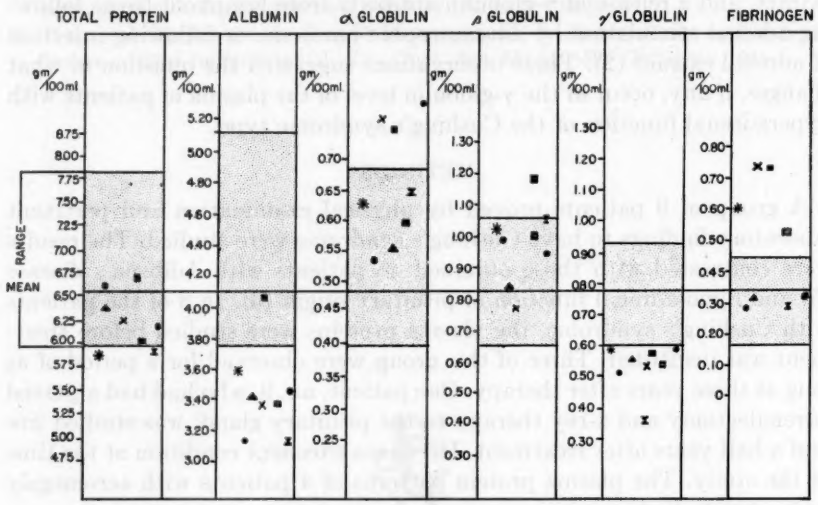

FIG. 1. Tiselius electrophoretic analysis of the plasma proteins of patients with Cushing's syndrome.

The plasma protein patterns of the 4 patients with acromegaly showed no consistent changes. Two of them who had moderately severe diabetes showed elevated β-globulin, similar to that found in patients with uncontrolled diabetes mellitus (4) without acromegaly.

DISCUSSION

The low plasma γ-globulin in these patients showing hyperadrenal cortical activity can be explained on the basis of the principles established by Dougherty, Chase and White (2) in experimental animals, namely, that administration of adrenal extract causes release of γ-globulin into the circulating plasma from lymphoid tissue. In the patient with hyper-

TABLE 1. CHANGES IN PLASMA PROTEIN PATTERN OF PATIENTS WITH CUSHING'S SYNDROME BEFORE, DURING AND AFTER THERAPY

Date	T.P.	A		α		β		γ		φ		Treatment and Clinical Conditions	Blood Pressure
	gm.	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%		
						Case Number 2							
5-11-43	5.49	3.11	56.6	0.54	9.3	1.00	18.3	0.52	9.4	0.32	5.9	Pretreatment	216/148
5-17-43 to 5-22-43		1200 r. total.										X-ray to pituitary	
6-22-43	6.57	3.90	59.4	0.66	10.0	1.25	19.1	0.39	5.9	0.37	5.6		185/140
6-21-43 to 6-26-43		1200 r. total.										X-ray to pituitary	
9-27-43													
10-12-43												Hemiadrenalectomy of both adrenals	
12- 6-43	6.27	3.54	56.5	0.48	7.6	1.14	18.3	0.63	10.1	0.48	7.6	Some nausea	160/110
10- 7-46	7.26	4.34	49.7	0.43	5.9	1.25	17.2	0.86	11.9	0.38	5.3	Condition excellent	
						Case Number 3							
4-20-43	6.38	3.37	52.8	0.88	13.7	0.82	12.9	0.56	8.8	0.75	11.8		180/120
4-12-43 to 4-22-45		1500 r.		6-22-43 to 6-27-43			2450 r.					X-ray to pituitary	
9-14-43, 10-10-43												Partial removal both adrenals	145/100
11-21-44	6.01	3.05	50.7	0.68	11.4	0.95	15.8	0.76	12.6	0.57	9.5	Looks and feels well, except for headaches	
10- 8-45	6.07	3.39	55.8	0.54	8.8	1.21	20.0	0.52	8.6	0.41	6.8	Looks normal except for obesity. Headaches have disappeared.	130/96
						Case Number 6							
6-14-43	6.02	3.32	55.2	0.63	10.5	1.01	16.7	0.53	8.8	0.53	8.8		
6- 4-44	6.07	3.28	54.1	0.47	7.7	1.01	16.6	0.88	14.5	0.43	7.1		
2- 5-46	6.89	3.40	49.5	0.74	10.7	0.97	14.0	1.22	17.7	0.56	8.1		
						Case Number 7							
1- 5-38												Hemiadrenalectomy	
6-29-43	6.49	3.57	55.0	0.58	8.9	0.87	13.4	0.87	13.4	0.60	9.3	Excellent condition	130/92
	Normal (Average of 25 normals)												
	6.51	4.09	62.7	0.47	7.2	0.81	13.1	0.77	11.7	0.33	5.4		

adrenal cortical activity the initial change could be imagined to be a release of γ-globulin. At the time the studies reported here were made, the stimulation had been of such long duration that the γ-globulin reserves stored in the lymphoid tissue had been exhausted, and so the level in the circulating plasma could not be maintained. The blood lymphocyte count, in the 4 cases in which it was determined at the time of the protein study,

TABLE 2. PLASMA PROTEIN PATTERN (TISELIUS ELECTROPHORESIS) IN ACROMEGALY

Patient Number	T.P. gm./100 ml.	A		α		β		γ		φ	
		gm./100 ml.	%	gm./100 ml.	%	gm./100 ml.	%	gm./100 ml.	%	gm./100 ml.	%
10	6.22	3.91	62.6	0.38	6.0	0.94	15.3	0.63	10.0	0.36	6.1
11	6.22	3.69	59.3	0.67	10.8	0.87	13.9	0.76	11.3	0.29	4.7
12	6.50	3.91	60.2	0.36	5.5	1.07	16.5	0.70	11.1	0.44	6.7
13	6.58	3.57	54.2	0.61	9.3	1.28	19.5	0.72	10.9	0.40	6.1

was very low, ranging from 615 to 2,000 per cu. mm. In the patients with hypoadrenal activity, either primary or of pituitary origin, the γ-globulin level was increased or at the extreme upper limits of normal. In these cases the γ-globulin reserves were adequate.

The marked increase in plasma protein level, especially in the γ-globulin fraction, following administration of testosterone (case 6) was interesting and agrees with the findings of Albright (1) which showed that administration of testosterone results in anabolism of protein. Protocols of the four patients who were observed over a period of years are appended.

Patient No. 2. The patient was first seen three years ago, then aged 24. He complained of arterial hypertension which he knew had existed for two years. He developed some degree of weakness, marked dryness of the skin, ease of bruising, striking roundness and redness of the face. Significant findings on examination included an appearance typical of Cushing's syndrome. Weight 146 pounds; height 63″; blood pressure 216/148. Blood count showed 5.5 million red cells; hemoglobin 13.5 Gm. Total blood volume was normal, showing 29 cc. of cells per kilogram. Water excretion test of Robinson, Power and Kepler showed an index of 21. Glucose tolerance was as follows:

	Fasting	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Blood sugar	92	206	248	145	106	58
Urine sugar	0	—	0	Tr.	Ft. Tr.	0

17-Ketosteroids estimated on a pure ketonic fraction, totalled 6.9 mg. per 24 hours. X-ray revealed no enlargement of the sella turcica. There was moderate decalcification of

the bones of the skull; slight decalcification of the spine and pelvis and evidence of osteoarthritis of the hip and some calcification in the pelvic vessels. In September and October 1943, bilateral adrenal exploration was done. No tumor was found in either adrenal and a portion of each gland was removed. The portions of glands removed measured 1.3 by 0.7 cm. and 1.1 by 1.2 cm. respectively. No microscopic abnormality was found. In general his improvement has been slow but steady. Adrenal insufficiency was seriously entertained as a diagnosis during the latter part of 1943, in spite of the fact that his blood pressure had never attained normalcy. When last seen, in October 1946, his appearance was entirely normal. He was symptom free, working daily on the highway and on a farm. Blood pressure remained 180/130. There has been a gradual shortening of the right leg associated with the shortening of the neck of the femur and considerable absorption of the head and neck, associated with marked deposition of new bone. This change is apparently due to progressive aseptic necrosis plus osseous hypertrophy. Similar changes of milder degree are present in the opposite hip joint. The previous appearance of decalcification of the spine has almost completely disappeared.

Patient No. 3. Symptoms of this 22 year old woman were first noted 4½ years ago. These were chiefly increasing weakness, headaches and blurred vision. At the time of our first examination 3½ years ago, she presented the typical appearance of Cushing's syndrome with adiposity of the trunk, face and neck. Hypertrichosis, with a rather fine type of hair, involved the face, abdomen, shoulders and to some extent the breast area. The face was markedly round and red. She had been amenorrheic for three years. Typical purple striae were present. Blood pressure was 160/100. Red blood count was 5.29 million; hemoglobin 13.5 Gm. Spine by x-ray appeared to be osteoporotic. 17-ketosteroids were 2.9 and 3.8 mg. in 24 hours on two occasions. Basal metabolic rate varied from approximately −15% to −18%. Glucose tolerance was as follows:

	Fasting	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Blood Sugar	111	193	292	246	238	122
Urine sugar	0	—	0	.33	.41	.40

X-ray therapy to the pituitary in doses of 2450 roentgen units delivered to each temple was given in April 1943 and since little or no improvement could be detected by September, 1943, bilateral partial adrenalectomy was performed. Considerable improvement followed this. Improvement, however, was very slow and for three years after operation, her weight was ten pounds greater than it was just prior to surgery, the weight being 174½ pounds; height 63″. Spine had become much less osteoporotic. Blood pressure three years postoperatively was 145/100.

	Fasting	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Blood sugar	96	139	161	161	157	89
Urine sugar	—	—	—	—	—	—

Patient No. 6, a white male, age 40 in 1946, has been under observation since January of 1937. His appearance is typical of that seen in the classical Cushing's syndrome. Outstanding features of the disease include a round red face with extremely plethoric appearance. He had persistent arterial hypertension, blood pressure usually being about 160/110. Typical purple striae were present. Red blood count has been as high as 6.02 million. X-rays of the spine and skull showed considerable demineralization as well as evidence of 15 rib fractures, all of which had been painless. X-ray therapy to the pituitary area was given in 1937 and in 1943 with little or no improvement. Therapy has included diet containing at least 100 Gm. protein per day and since 1943 protamine zinc insulin

15 to 20 units daily, as well as testosterone propionate 25 mg. two or three times weekly. In October of 1946, urinary corticoids measured by the method of Lowenstein and Corcoran were 23.2 mg. (Normal excretion is approximately 6 to 10 mg.) In November 1946, urinary 17-ketosteroids, measured on a pure ketonic fraction, were 23.9 mg. (Normal 7 to 14 mg.). In October of 1946 the glucose tolerance was as follows:

	Fasting	½ hr.	1 hr.	2 hr.	3 hr.	3 hr.
Blood sugar	112	216	272	316	208	157
Urine sugar	0	—	0	.42	.46	0

X-ray of the thorax and spine showed less demineralization than it had several years previously. Plasma potassium and sodium were normal. Blood urea was 45 mg.%, calcium 10.2, phosphorus 3.0 and cholesterol 191. The red blood count was 5.51 million; hemoglobin 13.5 Gm.

Patient No. 7. In 1937 this 26 year old white man was seen because of extensive epidermophytosis and onychomycosis. Two months previously and very suddenly severe pain occurred in the region of the lumbar spine, coming on while lifting. His appearance was typical of Cushing's syndrome and the history suggested that this appearance had developed over a period of the preceding 14 years. Glycosuria had been found on several occasions. His blood pressure was 188 systolic, 130 diastolic, height 67″ and weight 165 pounds. The skull was demineralized, the vertebral column was markedly demineralized with a compression fracture of one vertebral body. Glucose tolerance test showed mild diabetes. Serum calcium, phosphorus, fasting blood sugar, cholesterol, urea were normal on repeated occasions. The blood count showed 5,550,000 red cells and 14.0 Gm. hemoglobin. Assays for androgens done by the capon comb growth method on four occasions were normal, varying between 33 and 65 international units in 24 hours. Perirenal air injection showed evidence suggestive of enlargement of the right adrenal. In January of 1938, exploration of the right adrenal gland showed it to contain a small adenoma and this gland was removed. In March of that year, he was also given 800 roentgen units of x-ray to each temple. His gradual improvement was interrupted by the development of a urinary calculus which was removed by ureteral meatotomy in August of 1939. In November 1939 his weight was 143 pounds, blood pressure 128/92. It was necessary for him to wear a back brace for about a year following surgery, after which this was discarded and he is apparently completely well. Plasma protein studies were done approximately four years after adrenal surgery.

SUMMARY

Electrophoretic analysis of the plasma proteins of eight patients with hyperadrenal cortical activity (Cushing's syndrome type) showed low plasma albumin and γ-globulin level.

In two of the four cases studied following therapy, the plasma protein pattern tended to become normal. The plasma protein pattern of four patients with acromegaly was normal.

ACKNOWLEDGMENT

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THE CO-EXISTENCE OF HYPERTHYROIDISM AND PREPUBERAL EUNUCHOIDISM IN A MALE

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THE problem of the interrelationships between the thyroid gland and the gonads has been the subject of considerable study and controversy. Most of the reports concern themselves with the effect of thyroid activity on the gonads and only a rare reference has been made to the opposite relationship. These changes in gonadal function in the female have been reported frequently because menstrual disturbances lend themselves easily to clinical observation. Presumptive gonadal changes in the male have received much less attention and few reports have been published.

Not all observers believe that a direct relationship exists between these two endocrine organs. Van Dyke (13) concluded that thyroid activity probably has no significance in the regulation of gonadal function. Moore (9) stated that experimental data had not yet established the existence of any direct relationship between the thyroid and the testes although there was good evidence of the occurrence of indirect functional associations mediated probably by way of changes in oxygen metabolism. Salter (11) suggested 3 possible types of thyro-gonadal reciprocities:

(1) a peripheral sensitization of tissues to the gonadal hormones by the thyroid;
(2) effects produced by thyroid activity upon the gonadopituitary axis, or vice versa;
(3) indirect effects of the thyroid upon the gonads via alterations produced in glands other than the pituitary, i.e. the adrenals.

Among the infrequent references to the effect of the gonads on the thyroid gland is that of Kinsell, Hertz and Reifenstein (4). They studied the effects of testosterone propionate and methyl testosterone in several thyrotoxic patients in whom weight loss and metabolic functions were prominent features. The testosterone propionate decreased creatine excretion, increased nitrogen retention, increased the patient's well being and weight without significantly affecting the basal metabolic rate. Methyl testosterone, on the other hand, aggravated the thyrotoxic state by virtue of its calorigenic action and its effect in increasing creatine excretion. From this study alone one could not conclude whether native androgenic substances are helpful or deleterious in thyrotoxic states because our knowledge of the metabolism of androgens in disorders of the thyroid gland is meager. It would be pertinent to know whether methylated androgens play any part in the intermediary metabolism of androgenic substances in

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patients with thyrotoxicosis. Since liver functions are frequently altered in thyrotoxicosis, it may be no surprise if, as a consequence, there were changes in steroid metabolism as well.

One would anticipate from Marine's theory of Graves' disease that the clinical administration of androgenic material would aggravate the thyrotoxic state. Marine (5) has long considered the hyperactivity of the thyroid

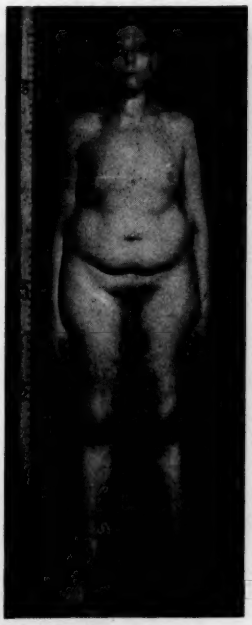

FIG. 1. Note the typical eunuchoidal measurements, hairlessness and extremely small external genitalia. Also note the stare.

gland in Graves' disease to be secondary to and dependent upon a primary anterior pituitary-gonad hormonal defect or anomaly which renders the individual's autonomic control over various visceral functions susceptible to injury by a great variety of non-specific influences. It is Marine's hypothesis that the gonadal disturbance consists of an imbalance in the androgen-estrogen ratio of the host favoring the preponderance of the androgenic function. In other words, there is presumed to be an absolute or a relative increase of androgenic over estrogenic substances. This androgen predominance stimulates the thyroid gland to produce more thyroid hormone which in turn tends to depress androgen production by its general depressing action on the anterior pituitary gland. The striking increase in

the incidence of Graves' disease at the menopause; the characteristic preservation, and at times hypertrophy, of the interstitial cells of the testes in Graves' disease in contrast to their involution in myxedema; the effect of successful iodine therapy in depressing the urinary excretion of androgenic material in true Graves' disease (6) plus the fact that no one had yet reported a case of Graves' disease in a male castrate (6) gave credence to Marine's hypothesis on the pathogenesis of hyperthyroidism. In the light

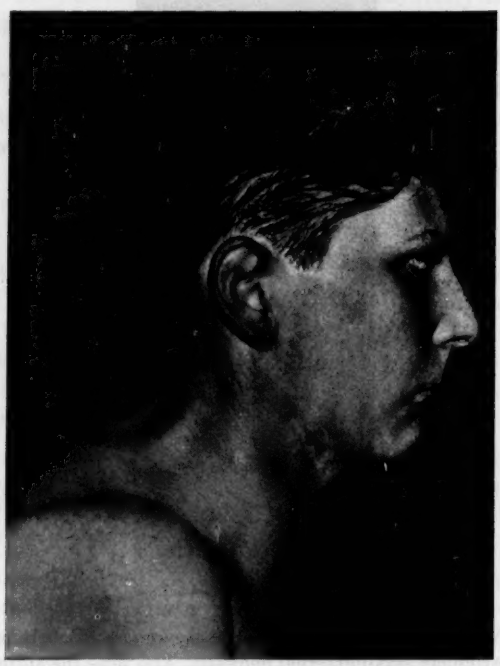

FIG. 2. Note enlargement of the thyroid gland.

of this theory the following report of a case of Graves' disease which developed in a typical prepuberal eunuchoid male is very interesting. In a search through the *Quarterly Cumulative Index Medicus* for the past 20 years, there is not a single reference to the co-existence of these two clinical syndromes.

CASE REPORT

History: The patient, a 28 year old white male, was admitted to the Beth Israel Hospital (#169228) on December 29, 1944 complaining of swelling of the neck of one year's duration, weakness and dizziness of one year's duration, and precordial and left elbow pain of 2 months' duration. About one year before admission the patient noted the

insidious onset of anterior swelling of the neck associated with an annoying, heavy and bounding pulsation. He also noted free and profuse perspiration, a feeling of warmth even in cold weather, palpitation, weakness, dizziness, tremor of the hands and increasing emotional instability. In the past year his weight had fallen from 170 pounds to 149¼ pounds at the time of hospitalization. Precordial discomfort associated with some pain in the left elbow set in 2 months ago but these were not distressing enough to warrant medical attention at the time. For several months he had noted moderate dyspnea on moderate exertion but no orthopnea.

The patient's story was not completely reliable for he tended to change its context with every interrogation by members of the hospital staff. One of his private physicians

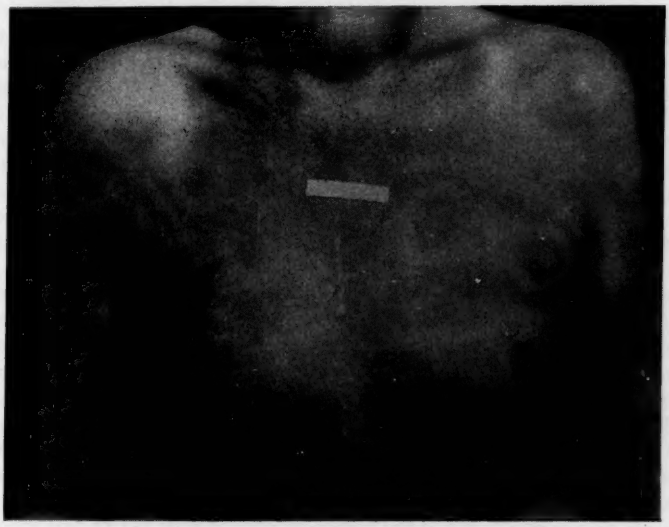

FIG. 3. Note the bilateral gynecomastia.

offered the information that the patient showed full evidences of hyperthyroidism with a tachycardia of 145 beats per minute when he was first consulted in 1943. On one following occasion the patient exhibited an episode of transient auricular fibrillation. His physician reported that the patient gave the history of having taken one thyroid tablet 3 times a day "for some time for the purpose of weight reduction." In the hospital, the patient denied having taken this drug steadily but admitted having ingested thyroid occasionally. The above mentioned physician also noted his bizarre general appearance which he interpreted as a Frohlich's Syndrome and treated him with a Parke Davis preparation of combined gonadotropic and pituitary hormone by injection twice weekly for 1½ months after which the patient ceased contact with the doctor. The only medications used to treat his hyperthyroid state were mild sedatives.

The past history included the congenital existence of undescended testes with an extremely small phallus and scrotum. He had never shaved. His body growth was rather rapid, especially his lower limbs. The voice was always high pitched and he admitted

finally that his libido was nil in spite of frequent falsification of this fact to several historians. The only treatment he ever received for this condition was that reported above by his physician. The treatment effected no change in his physical status. At the age of 10 he had a bout of rheumatic fever with polyarthritis and carditis for which he was treated at Bellevue Hospital at the time. There was no history of chorea, nor congestive heart failure and there had been no recurrences of rheumatic fever.

Family History: His mother had high blood pressure. Otherwise negative.

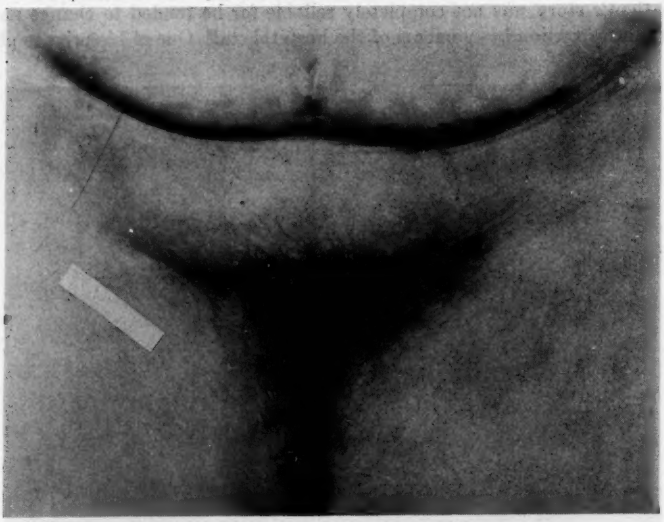

FIG. 4. Note the extremely small external genitalia and the few suprapubic hair follicles.

Physical Examination: The patient was a well nourished white male with a somewhat flushed, anxious expression. His body contour was feminine in appearance. The skin had a waxy smooth cast, was moist and hairless except for a few suprapubic hair follicles. The voice was high pitched. The eyes exhibited a distinct stare, slight bilateral exophthalmus, slightly widened palpebral fissures and a lid lag. The neck revealed a diffusely enlarged, firm, bulging thyroid gland over which could be heard a systolic bruit. The lungs were clear. The point of maximum cardiac impulse was visible and palpable in the sixth intercostal space outside the mid-clavicular line. The heart sounds were of good quality. The rate was regular and rapid at 130 beats per minute. A2 was louder than P2. Double apical and basal murmurs were audible. The blood pressure ranged between 110–130 mm. systolic and 60-0 mm. diastolic. A bilateral gynecomastia of moderate degree was present. The abdomen was negative. The extremities showed the measurements typical of prepuberal eunuchoidism. The distance from the symphysis pubis to the feet was 36 inches compared to the measurement of 32½ inches from the top of the head to the symphysis pubis. The palms were moist and warm. The deep reflexes were exaggerated and there was a fine tremor of the outstretched hand. A slight to moderate degree of thoracic kyphosis was evident. The phallus was extremely small. The testes were

undescended and could not be palpated in the inguinal canals. The scrotum and prostate gland were also extremely small.

Laboratory Data: Urine was negative. The red blood cell count was 4.41 million per cubic millimeter and the hemoglobin was 11 grams. The white blood cell count was 7,700 with 64 polymorphonuclears, 1 basophil, 28 lymphocytes and 7 monocytes. The Wassermann, Kline and Kahn tests were negative. The non-protein-nitrogen level of the blood was 33 milligrams per cent. An oral glucose tolerance test revealed a fasting blood sugar level of 96 milligrams per cent followed by levels of 122, 96, 80 and 78 milligrams per cent at hourly intervals after the ingestion of a single dose of 100 grams of glucose. The serum cholesterol before any treatment was instituted ranged between 120 and 130 milligrams per cent on 3 occasions. The creatine excretion in urine was 255 milligrams in 24 hours. The creatinine excretion in urine was 1,253 milligrams in 24 hours. The total serum protein was 4.18 grams per cent with serum albumen of 2.49 grams per cent and serum globulin of 1.69 grams per cent. The insulin tolerance test showed a fasting blood sugar level of 98 milligrams per cent with one-half hourly readings after 7 units of insulin intramuscularly (0.05 unit per pound of body weight) of 96, 63, 66 and 86 milligrams per cent. The visual fields were normal. The electrocardiogram was normal. The x-ray of the skull was normal. The sella turcica was within normal limits. The 17-ketosteroid excretion in the urine was reported* as alpha fraction 2.7 milligrams in 24 hours (normal 8–15 mg.) and beta fraction 0.07 milligram in 24 hours (normal 0.3–4 mg.). On January 6, 1945, the blood total iodine content* was 8.4 micrograms per cent (normal 2–4).

Course: On January 29, 1945, treatment of the patient's hyperthyroidism was started with the initial daily dose of 1.2 grams of thiouracil and 150 mg. of pyridoxine. Clinical improvement was rapid and dramatic. As his condition improved, the dosage of thiouracil was decreased to 0.2 gram daily which was the dosage at the time of his discharge from the hospital on February 20, 1946. The fall in the basal metabolic rate paralleled the disappearance of the signs and symptoms of thyrotoxicosis. The weekly BMR determinations before institution of thiouracil were plus 35, plus 36, plus 42 and, following treatment, plus 44, plus 31, plus 9 and minus 3 per cent at the time of discharge from the hospital. His weight which diminished during hospitalization from 149¼ pounds to 144½ pounds rose to 147 pounds after thiouracil treatment. There was no change in the total leucocyte or differential blood count while in the hospital. He was referred for follow-up to the Metabolic Clinic of the hospital. There his co-operation was very poor, his attendance erratic and his behavior delinquent. In April 1945, while under thiouracil therapy he developed a moderate granulopenia which soon reversed itself on cessation of therapy. He refused to re-enter the hospital for observation and change in the form of therapy. Nothing was heard of him until he returned to the medical clinic on September 24, 1945 and again on October 15, 1945, showing auricular fibrillation and marked thyrotoxicosis. His blood picture was within normal limits. He was again urged to enter the hospital but again refused. No further information about his status was obtained until it was learned that he died at home in March 1946. Communications with his family physician revealed that his course was rapidly downhill. The signs of thyrotoxicosis became more marked and auricular fibrillation became chronic in September 1945. His weight declined rapidly until he developed marked congestive heart failure which was unaffected by digitalis and dehydration measures. Necropsy examination was not performed.

* The 17-ketosteroid and blood iodine determinations were performed by Dr. E. J. Baumann of Montefiore Hospital, New York City.

DISCUSSION

From both a clinical and laboratory viewpoint, it is evident that this individual presented the co-existence of two endocrinopathic syndromes, namely eunuchoidism and hyperthyroidism. This combination of glandular disturbances has not been reported in the literature heretofore. In several studies of eunuchoid males it was found that the basal metabolic rates were always below normal or normal (3, 8). The usual range of the basal metabolic rates in eunuchoid males was −15 to −20 per cent with an occasional low level of −30 per cent (3, 8). The mechanism for this hypometabolic state in eunuchoidism is not fully understood. It may be due to the fact that with the marked decrease in androgenic material the sensitization of the peripheral tissues to thyroid hormone is diminished. This would be the counterpart of the increased sensitivity to thyroid extract exhibited by eunuchoids after treatment with testosterone propionate as described by Eidelsberg and Ornstein (2). Other possible explanations for this hypometabolic state are (a) the so-called crowding-out of the thyrotropic factor of the anterior pituitary by the increased production of gonadotropic substance when the eunuchoidism is primarily testicular in origin and (b) the presence of panhypopituitarism when the eunuchoidism is of pituitary origin.

In the light of our present knowledge, it is difficult to understand the pathogenesis of the association of hyperthyroidism and prepuberal eunuchoidism as presented above. If, as Aron and Benoit (1) believed, castration increased the production of the thyrotropic as well as the gonadotropic hormones of the anterior pituitary gland with consequent increased activity of the thyroid gland, one would expect the co-existence of Graves' disease and castration with greater frequency than actually exists. Moore (10), on the other hand, stated that no severe changes appear to develop in the thyroid gland after castration. McCullagh and Walsh (7) could demonstrate no change in the size of the thyroid gland in rats 85 days after castration.

The possibility exists that the patient we have just described had ingested exogenous thyroid substance to the point of permanent hyperthyroidism. Such an occurrence is, however, rare, particularly in syndromes characterized by a hypometabolic state such as exists in eunuchoids. Schoeller and Gehrke (12) reported that male castrate mice on a similar dose of thyroxine (0.5 mg.) showed a 50 per cent less rise in metabolism than normal mice. On the other hand, as stated above, Eidelsberg and Ornstein (2) found that there was an increased sensitivity to thyroid in eunuchoids who had been treated with testosterone propionate and definite hypermetabolic rates in eunuchoids without Graves' disease were reported

by McCullagh (8) after prolonged treatment with large doses of methyl testosterone. The patient reported here had never been treated with testosterone products for his eunuchoidism. The relationship of the superimposed Graves' disease to the underlying and antecedent eunuchoidism remains therefore a puzzle defying explanation with our present limited knowledge regarding the thyro-gonadal axis. It is greatly regretted that necropsy examination was not performed for it may have contributed to a clarification of the clinical pathological problem. One wonders what the adrenal cortex or perhaps the hypothalamus showed in this case and what relationship they may have had to the thyro-gonadal axis.

SUMMARY

The very unusual co-existence of hyperthyroidism and eunuchoidism in a male is reported. The relationship between the two clinical syndromes is unknown.

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USE OF MASSIVE DOSES OF VITAMIN A IN THE TREATMENT OF HYPERTHYROIDISM

A PRELIMINARY REPORT

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INVESTIGATIVE work during the last decade and a half has established the close interrelationships existing between hormones and vitamins in their effects on the same organs of the body (19). Of considerable interest is the relationship between thyroxine and vitamin A, concerning which an extensive literature has developed abroad but strangely enough comparatively little in this country. The antagonism between the thyroid hormone and vitamin A was first suggested a half century ago (39) and confirmed between 1930 and 1939 by extensive experimental work in animals.

For the present report the effects of massive doses of vitamin A (200,000 to 400,000 I.U. daily) in hyperthyroidism were tested in two patients. One was a woman with menopausal exophthalmic goiter of moderate severity complicated by essential hypertension; the second, a rather severely toxic hyperthyroid woman. Routine studies such as urinalysis, blood count, blood sugar and electrocardiogram were performed in both patients; the metabolic rate and blood cholesterol were determined as in Table 1; the weight and pulse rate were checked semi-weekly, as was the blood pressure, in *case 1*. For ease of administration the vitamin A was dispensed in divided dosage in capsules containing 50,000 I.U. each.[1]

CASE REPORTS

Case 1. Diagnosis: Menopausal exophthalmic goiter with essential hypertension.

Mrs. R. L., a white woman aged forty-six, an inspector of clothes, had been in good health until 1943, a year before she became our patient, when asymptomatic hypertension was discovered. The menses had been irregular for two years and during this time she suffered from mild vasomotor phenomena (flashes, sweats and chilliness). She had been married nineteen years and had one child alive and well. Appendectomy was performed in 1917.

Five weeks before she came to the hospital she had noticed marked progressive irritability and nervousness, tremors of the hands and increased perspiration. Her appetite was good but her weight had fallen during this period from 110 pounds to 105 pounds. Palpitation had been present for three weeks. During the last week she had suffered from

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[1] Vitamin A was furnished through the courtesy of Edward R. Neary, M. D., Director of the Professional Service Division, White Laboratories.

insomnia. A sensation of substernal pressure developed after dinner. She had been forced to leave her work at the onset of her symptoms.

Physical Examination: The patient was short and slender weighing only 105 pounds. Her skin was flushed and moist. Slight exophthalmos was present with definite lid lag. The thyroid gland was slightly diffusely enlarged, especially the right lobe, and was smooth. The pulse-rate ranged between 100 and 120 per minute. The heart sounds were booming. The heart and lungs were normal. The abdomen revealed a right lower quadrant scar. A fine tremor of the hands was present. The reflexes were normal, as was sensation.

Laboratory Data: The basal metabolic rate was +44 and the blood cholesterol 160 mg. per cent. Urinalysis was normal. The blood count revealed hemoglobin 13.0 Gm.,

TABLE 1. CORRELATION OF BASAL METABOLIC RATE AND BLOOD CHOLESTEROL WITH TREATMENT BY VITAMIN A AND LUGOL'S SOLUTION

Duration Treatment (weeks)		Basal Metabolic Rate	Blood Cholesterol (mg. %)	Vitamin A (I.U. daily)	Lugol's Solution (minims daily)
Case #1	control	+44	160	300,000	0
R.L.	4½	+12	300	300,000	0
	11	+ 6	260	300,000	0
	14½	—	—	0	0
	17½	0	330	0	0
	19½	—	—	200,000	0
	24½	− 1	296	200,000	0
Case #2	control	+53	175	400,000	0
L.Q.	3	−11	190	400,000	0
	5½	+ 4	199	400,000	0
	19	+ 7	269	400,000	0
	32	− 2	266	400,000	0
	47	—	—	300,000	10
	51	+ 6	223	300,000	10

red blood cells 4,300,000/c.mm., white blood cells 6,200/c. mm., with neutrophils 68 per cent, lymphocytes 20 per cent, and monocytes 12 per cent. The blood sugar was 105 mg. per cent. The electrocardiogram was normal. X-ray showed no defects in the heart or lungs.

Progress: The administration of 300,000 I.U. daily of vitamin A was begun June 30, 1944 (Table 1). After one week the pulse-rate was 108/minute and severe headache was present, but the feeling of nervous tension had disappeared. Insomnia was still present. After three weeks' treatment the pulse rate had fallen to 88/minute, the weight had increased three pounds and all symptoms, except mild flashes and substernal pressure after dinner, had disappeared. A week later the patient was totally asymptomatic, the basal metabolic rate had fallen to +12, the blood cholesterol had risen sharply to 300 mg. per cent and the weight was stationary. Steady improvement continued thereafter

with further reduction in pulse rate to 66/minute, progressive increase of weight to 120 pounds, continued fall of basal metabolic rate, maintained increase of blood cholesterol and a progressive diminution in the size of the thyroid gland until the fourteenth week of treatment when vitamin A therapy was discontinued. At this time the patient resumed her work. The thyroid gland was now entirely normal to palpation.

Vitamin A administration, 200,000 I.U. daily, was resumed after a lapse of five weeks for the treatment of the persistent hypertension and was discontinued ten weeks later. The basal metabolic rate continued to fall, as did the pulse rate, and the blood cholesterol remained at approximately the same level. The blood pressure fell from an average of 188/119 to 148/101. The patient has been under continuous observation for thirty-six months since treatment was first begun and has remained entirely normal except for a moderate hypertension. During the last thirty months she has not received vitamin A.

Case 2. Diagnosis: Hyperthyroidism

Mrs. L. Q., a white thirty year old housewife had, four years prior to her present illness, exhibited irritability for two months, tachycardia, a slight weight loss, marked tremors of the hands and a marked, diffuse, soft enlargement of the right lobe of the thyroid gland. The basal metabolic rate was +22 and the resting pulse rate 88/minute. She had been treated four weeks with Lugol's solution, minims 30 daily, with resultant quiescence of symptoms. Her past medical history was otherwise negative.

At the time of her admission to the hospital, June 16, 1945, her symptoms were extreme nervousness, palpitation and a weight loss of eight pounds in four weeks despite a ravenous appetite. Her symptoms seemed to date from the shock of her husband's induction into military service.

Physical Examination: The patient was of medium height and slender. The temperature was 99° F., the blood pressure 104/70, the weight 101 pounds, and the pulse rate 108 to 140/minute. The entire thyroid gland was diffusely moderately enlarged and firm. The right lobe felt slightly nodular. The skin was moist. No ocular signs were present. She exhibited fine tremors of the hands. Examination was otherwise essentially negative.

Laboratory Data: The metabolic rate was +53 and the blood cholesterol 175 mg. per cent. Urinalysis was normal. The blood count revealed hemoglobin 12.8 Gm., red blood cells. 4,400,000/c.mm. and white blood cells 6,500/c.mm. with normal differential. The blood sugar was 92 mg. per cent. The electrocardiogram was normal. X-ray showed a normal chest.

Progress: Treatment was begun with 400,000 I.U. of vitamin A daily. The basal metabolic rate (Table 1) plummeted to −11 after three weeks of treatment and the blood cholesterol increased slightly to 190 mg. per cent. The pulse rate fell to 80, and the tremors of the hands became less marked. The goiter became definitely smaller. She felt improved, with a marked decrease of nervousness and irritability. After five and a half weeks of treatment the basal metabolic rate was +4, and the blood cholesterol 199 mg. per cent. The pulse rate was 116, an elevation apparently due to premenstrual tension (as will be discussed below). After seven weeks' treatment the goiter had regressed greatly in size, was still diffuse but no longer nodular. Thereafter her course continued uphill with slow but progressive improvement in pulse rate and weight, as well as a continuance of normal basal metabolic rate and further increase in blood cholesterol values. The smoothness of her course thereafter was interrupted only by symptoms of premenstrual tension.

After forty-two weeks of treatment the pulse rate showed persistent elevation (86 to 96/minute) from its preceding lower levels, but the patient felt well, maintained her improvement and showed no signs or symptoms of toxicity. However, in an attempt to forestall any possibility of a recrudescence of toxicity, Lugol's solution (10 minims daily) was added to the vitamin A therapy at the forty-seventh week. The iodine produced no additional effect on the basal metabolic rate (+6) or the blood cholesterol (223 mg. per cent) which was determined four weeks later, but did decrease the pulse rate to 78/minute, at which level it has remained. All medication was now discontinued. The patient has remained perfectly well during the twelve months she has been under observation since vitamin A therapy was discontinued. Her weight reached 111 pounds (a gain of 10 pounds). The thyroid gland is barely palpable, and the patient appears to be entirely cured of her hyperthyroidism.

Hyperthyroidism

Proof of the antagonism between vitamin A and the thyroid hormone rests on an extensive laboratory and clinical background. The use of thyroxine prevents the production of hypervitaminosis A in animals (20). Hyperthyroidism in rats can be counteracted by a diet rich in vitamin A and B (2), or by cod-liver oil (60). Soskin and Mirsky (50) cured a very toxic hyperthyroid patient by use of a high fat diet (230 Gm. of fat daily). Vitamin A counteracts the rise in basal metabolism produced by large doses of thyroxine and ameliorates the toxicity (3, 31, 38, 57).

Abroad, vitamin A has been used successfully in the treatment of human hyperthyroidism. In Germany, Wendt (57, 58) achieved excellent results in six patients with moderately severe to severe hyperthyroidism by the use of 150,000 to 240,000 I.U. of vitamin A daily over periods of time ranging up to twelve weeks. Falta (21) reported a cure in a patient with markedly toxic hyperthyroidism. Dietrich (13) obtained excellent results in five of six patients treated for several weeks; the concurrent use of iodine seemed to enhance the therapeutic results obtained. Goth (26) reported good results in six of seven cases treated for several weeks with 100,000 to 200,000 units daily. Tislowitz (52) cured one patient by the administration of three to four glasses of carrot juice daily.

In France vitamin A therapy has been utilized only on a rather small scale. Sendrail (48) used small doses of vitamin A (2400 I.U. daily; 25,000 I.U. daily in one case only) combined with Lugol's solution and secured promising results in the majority of patients. Azerad (5, 6) used 120,000 I.U. daily in a large number of cases and obtained complete success in the majority, but only after prolonged treatment. In a second group which had been treated successfully with iodine or vitamin A alone the symptoms recurred after cessation of treatment; when vitamin A was combined with iodine the results were excellent. In a third, rather small, group the combination of vitamin A and iodine proved a complete failure. Rymer (43)

secured much more rapid improvement with vitamin A plus iodine than with the use of iodine alone. In general, the Germans used a much higher dosage of vitamin A comparatively than the French did.

Elsewhere abroad Mariante (33), employing 180,000 to 240,000 I.U. of vitamin A daily, obtained good results in eight of fourteen cases. Casassa and Pescarmona (10) secured relief in hyperthyroidism with moderate dosage of vitamin A.

The striking feature in the present series is the rapid fall of the basal metabolic rate in *cases 1* and *2*, being extremely precipitous in the latter case and approaching that ordinarily obtained with the use of iodine. The successful cases reported in the literature showed that the basal metabolic rate usually required at least seven weeks to be restored to normal. Many authors, especially the French (6), emphasize that several months may be required for the basal metabolic rate to fall to normal levels. In all probability the more rapid results obtained in this series may be ascribed to the relatively much larger dosage employed.

Keeping pace with the fall in basal metabolic rate was the decrease in pulse rate which fell to normal levels within three weeks in both cases. As striking was the amelioration of symptoms, being definitely pronounced within one and a half to two weeks in both cases. The weight was much slower in attaining its maximum and varied with each case insofar as rate of increase was concerned. In *case 1* the weight began to climb only at the end of seven weeks' treatment. In *case 2* the weight showed very little increase until thirty-two weeks had elapsed when it began to increase sharply coincident with a sudden pronounced fall in the pulse rate. We must conclude that subclinical toxicity was present in this patient until this point.

There is an increased need for vitamin A in hyperfunction of the thyroid gland (47), the blood content of vitamin A in human beings suffering from hyperthyroidism falling even to zero despite adequate intake (63). It is noteworthy that there exists a certain accord between the fall of blood vitamin A level and the augmentation of the basal metabolic rate. Treatment of the hyperthyroid patient with vitamin A produces an elevation of vitamin A in the blood serum that is indicative of improvement (4) and comparable with the rise of blood cholesterol (30). The fall of vitamin A in the blood serum has been advanced as a diagnostic aid in the determination of hyperthyroidism (51).

Within three to four weeks after treatment was begun in the present cases the goiter began to decrease in size. This is similar to the experience of Fasold (22). Regression then continued at an accelerated rate, with complete disappearance of the goiter in as little as fourteen weeks in one case.

Blood Cholesterol

The blood cholesterol value is low in toxic thyroid states (27, 28). The fall in cholesterol value in hyperthyroidism has been attributed to the chronic, toxic, interlobar, parenchymatous hepatitis which is present in 50 to 75 per cent of hyperthyroid patients (7). Wendt (59) showed that vitamin A produces a hypercholesterinemia, with some patients showing a rise to 250 mg. per cent or higher. An attempt has been made to show that vitamin A is the catalyst in the rise of the serum lipoids which follows vitamin A administration (59). Dzialoszynski (15) has pointed out that vitamin A occurs in human blood in some sort of association with a plasma protein (probably serum albumin), cholesterol and lipoids.

The blood cholesterol in the present series showed the same inverse relationship with the basal metabolic rate as follows the use of iodine in hyperthyroidism. The increase was very striking in *case 1*, showing a steady rise even two weeks after the use of vitamin A had been discontinued. What proportion of the rise in cholesterol was due to the fall in the basal metabolic rate and what proportion to the ability of vitamin A to produce hypercholesterinemia is not clear (59).

Vitamin A and Iodine

The synergism of iodine and vitamin A in the treatment of hyperthyroidism has been mentioned. In *case 2* the addition of iodine seemed to hasten the reduction of the pulse rate. During thyrotoxicosis the glycogen and vitamin A reserves of the liver are depleted (18, 56). A point of interest made by Schneider is that the use of vitamin A combats the fall in glycogen content of the liver so adequately that iodine can be dispensed with (44).

Effects of Vitamin A on the Sexual Mechanism

Vitamin A produces significant effects on the sexual mechanism, although our knowledge of the subject is rather meager (22, 34, 62). There is evidence to indicate the specific important role of vitamin A in the hypophyseal-gonadal interaction (54).

In *case 1* the menses were irregular before treatment was begun and showed no change under treatment. In menopausal hyperthyroidism we are concerned with excess of thyrotropic hormone (49). The probable mechanism whereby vitamin A was of value in this case may be by virtue of diminution of the thyrotropic hormone through direct action of vitamin A, or possibly through inhibition of the gonadotropic hormone which normally stimulates production of the thyrotropic hormone. *Case 2* had had regular menses every thirty-five days, lasting three to four days.

Under vitamin A treatment the menses tended to come closer together, ranging from twenty-six to thirty-four days. There was little, if any change in the flow. An interesting feature was that at the forty-seventh week of treatment, during the hot July weather, she developed numerous flashes and a sensation of chilliness of a mild character that became very slight in the course of four weeks and disappeared completely during the next week. These symptoms may be ascribed to a diminution in ovarian function.

Case 2 also displayed some of the group of symptoms now well known under the term "pre-menstrual tension" (61): increased subjective nervousness, restlessness, depression and crying spells. The symptoms began three days before, and ceased promptly with the onset of the menses. They became progressively less marked and finally disappeared completely after thirty-two weeks of treatment with vitamin A. The mechanism is open to question, possibly involving a decreased formation of circulating estrogen.

Action of vitamin A

The actual mechanism by which vitamin A is effective in the treatment of hyperthyroidism is still unknown. Several theories have been advanced. Spectroscopic methods have demonstrated that thyroxine and carotene *in vitro* are bound together, thereby inactivating the thyroxine (18, 24). There is no proof that such a direct chemical inactivation takes place *in vivo*. Schneider thought that the action was peripheral, protecting the liver against the action of thyroxine tending to lower the reserves of glycogen in the liver (45). Abelin felt that the antagonism between thyroxine and vitamin A was explainable by their opposing effects on the lipid metabolism of cells and the glycogen metabolism of muscle (3). Fasold and Peters, as well as Abelin, postulated a direct antagonism between vitamin A and thyroxine (1, 20). Others concluded that vitamin A has a direct inhibitory action on the complex hypophyseal thyrotropic hormone, preventing the liberation of thyroxine (17, 23). Belasco and Murlin concluded that possibly vitamin A, by virtue of its double bonds, is able to take up the available iodine in the body tissues and, either by acting on the thyroid gland or the anterior pituitary gland directly, affect the storage of colloid with consequently lowered oxygen uptake of the thyroid gland (8). McCarrison's conclusion that the thyroid gland is unable to deal with iodine in the normal manner in the absence of a sufficiency of vitamin A, with goiter resulting, is amply supported (12, 32, 35, 42).

Toxicity of Vitamin A

The effects of hypervitaminosis A in animals are well established. Trophic changes occur in the skin, bones and bone marrow. The skin

lesions, which appear first, consist of coarsening of the hair, ring crusts on the tail and inflammatory changes in the conjunctiva and nose (37, 62). The skeletal lesions consist of an atrophy of the long bones which become very thin and brittle, often resulting in spontaneous fractures (9, 11, 16). The fractures are due to a negative calcium balance with drastic decalcification of the long bones and vertebral column (9, 11, 41). There is a marked tendency to hemorrhage (36). Other pathology reported in animals includes focal liver necrosis, toxic degeneration of striated muscles and testis and a glomerulo-nephritis with calcification which is claimed as the usual cause of death (14).

In human beings the literature concerning the toxicity of hypervitaminosis A is rather meager. As long ago as 1596 it was recognized that polar bear and seal (Phocabarbata) liver could produce illness in man (loss of skin from head to foot) (40). Other expeditions proved this to be due to the extremely large value of vitamin A in polar bear and seal liver and found that toxicity develops in man with an ingestion of 7,500,000 I.U. of vitamin A daily (41). Schneider took a dose of 900,000 I.U. of vitamin A with the production of only light perspiration and a feeling of anxiety (46). Lehman and Rapaport used a single dose of 2,000,000 I.U. as a test for visual deficiency in children. They also prescribed 100,000 to 200,000 I.U. daily for months without discernible bad effects (29).

Note should be made of the extremely high dosage at which toxic effects begin in man. Until now the Germans used the highest dosage (up to 250,000 I.U. daily) without toxic effects; in the two cases reported here dosage as high as 400,000 I.U. daily over a long period of time was employed without ill effects. This lack of toxicity is to be expected when we compare even the massive doses used here with the relatively enormous doses required to produce illness in man (a safety margin of approximately 20 to 1). This is further attested by the lack of toxicity in a group of 100 hypertensive patients treated with similar dosage over periods of eighteen months.[2] The only other possibility of harmful effects rests with the occurrence of unusual sensitivity to vitamin A (25).

Normally no vitamin A is found in the stool (55) and never in the urine except under certain pathological conditions (53). No vitamin A is excreted in the feces under an ingestion of 25,000 I.U. daily; about 3 per cent to 4 per cent is excreted on a dosage of 76,000 I.U. daily, and an increasingly higher percentage as the intake is increased (55). Apparently the excess of vitamin A is rapidly destroyed in the body. From the data presented here it is permissible to conclude that even massive doses of vitamin A may be safely used in human beings over periods of many months.

[2] Work to be published by author.

SUMMARY AND CONCLUSIONS

1. Extensive experimental work in animals and clinical work in human beings in Germany, France, England and elsewhere abroad during the last seventeen years has proved conclusively the antagonism between vitamin A and the thyroid hormone, as well as the great value of vitamin A in the treatment of human hyperthyroidism.

2. Vitamin A was used in two cases of hyperthyroidism in massive doses (200,000 to 400,000 I.U. daily) for periods up to fifty-one weeks.

One case of menopausal exophthalmic goiter with hypertension, treated fourteen weeks, showed complete cure of the hyperthyroidism; continuous observation for thirty months subsequently has revealed no recrudescence. One case of hyperthyroidism with severe toxicity, treated fifty-one weeks, resulted in complete cure; continuous observation for twelve months subsequently has revealed no recrudescence.

3. The thyroid enlargement regressed within three weeks and disappeared within fourteen weeks. The slight exophthalmos which was present in one case disappeared completely under treatment.

4. Vitamin A acts very much like iodine in producing a rapid decrease in the basal metabolic rate, increase in the blood cholesterol and marked amelioration of symptoms, but a slower decrease of pulse rate and increase in weight.

5. The addition of iodine to vitamin A therapy may enhance the effectiveness of vitamin A.

6. Vitamin A produces significant effects on the sexual mechanism, especially ovarian function. Vitamin A produced a cure of the symptoms of pre-menstrual tension in one patient.

7. A marked maintained fall in blood pressure was obtained in the patient with menopausal exophthalmic goiter and hypertension.

8. The mechanism by which vitamin A is effective in the treatment of hyperthyroidism is still a matter for speculation.

9. Vitamin A is innocuous, even in doses of 400,000 I.U. daily over periods ranging up to fifty-one weeks.

10. Vitamin A is a promising therapeutic agent in the treatment of hyperthyroidism, both for cure and for alleviation of the toxicity.

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GYNECOMASTIA

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GYNECOMASTIA was first described by Basedow in 1848 (26). Since that time isolated reports of this condition have appeared in the literature. In 1938 Glass and Bergman (12) demonstrated that the ratio of the urinary androgens to the urinary estrogens in patients with gynecomastia was lower than normal, approaching the ratio found in females.

In 1942, Klinefelter, Reifenstein and Albright (20) presented a syndrome characterized by gynecomastia, aspermatogenesis, aleydigism and increased urinary excretion of the follicle stimulating hormone. They discussed the possible endocrine relationship in its etiology. Investigating similar cases in 1945, Heller and Nelson (15) showed that gynecomastia was not a constant sign of the syndrome and suggested a different etiology for the enlarged gland.

During World War II gynecomastia assumed the aspect of an occupational disability in the Armed Services because of the mechanical irritation of the enlarged gland and the psychic embarrassment resulting from communal living conditions.

The term, gynecomastia, refers to a unilateral or bilateral enlargement of the male breast which occurs as a result of hyperplasia of duct epithelium and periductal stroma. The term is not used in reference to those enlargements of the male breast occurring as a result of neoplasm, infections, cysts, and increased adipose tissue. The last named condition is referred to as pseudogynecomastia (11).

INCIDENCE

Webster (28) reported an incidence of 6.96 to 8.69 cases of gynecomastia per 100,000 Navy personnel during the years 1939 to 1942 inclusive.

A direct communication to us from the Army Service Forces, Surgeon General's Office, Washington, D. C., states: "The admission rate for gynecomastia in the United States Army is about 13 per hundred thousand per year."

In 1943, while prisoners of war, Major Robert Lewis (30) of the United States Army Air Forces and Colonel W. D. North of the United States Army Medical Corps observed among approximately 3,200 American prisoners of war who had spent nearly one and one-half years in Japanese

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prison camps, numerous men with a single, quarter-sized, smooth, subareolar nodule in the breast. These lesions were unilateral or bilateral. A survey of all personnel in one day by Major Lewis revealed an incidence slightly over four per cent. Many of these nodules disappeared spontaneously. Microscopic studies were not possible at the time.

The pathology laboratory's records of the United States Army medical installation, Brooke General Hospital (31), show that 72 cases, called gynecomastia by the pathologist, were operated on in 1945 and ten cases during the first three months of 1946. Grossly, many of the cases were reported as a nodule in the breast, while an equal number were listed as a diffuse enlargement. During 1945 the records of the surgical section (32) at Brooke General Hospital listed a total of 7,281 operative procedures being performed.

ETIOLOGY

A multiplicity of factors have been cited as the cause of gynecomastia. The etiology is frequently obscure. Inasmuch as normal breast hypertrophy in the female has been demonstrated to be the result of the action of the ovarian hormones, especially estrin (2, 27), it is natural to think of estrogenic hormones when considering mammary gland enlargement in the male. However, as demonstrated by Corner (2), pituitary injections alone have produced mammary gland hypertrophy in ovariectomized virgin rabbits.

The general consensus revealed by a review of the literature is that gynecomastia is a result of an altered endocrine status. Quantitative estrogen and androgen tests made on the urine of a number of patients with gynecomastia showed the A:E (androgen:estrogen) ratio to be altered substantially. Glass and Bergman (12) found with their methods an average urinary A:E ratio of 15.3 for normal males and 2.3 for normal females; the A:E ratio in their two cases of gynecomastia was consistently around 5.0.

Menville (22) feels that mechanical stimulation or irritation may play a very important role in male breast hypertrophy; yet he was able to obtain a history of actual trauma in only 14.5 per cent of his cases. Other investigators have recorded a similar lack of evidence for the mechanical irritation theory. It is difficult to determine whether the condition is a result of trauma or made noticeable by trauma. The high incidence in the army is felt by some to be the result of training or combat. The prolonged wearing of straps and packs is considered the greatest contributory factor.

Trauma may be an associated factor in individuals undergoing hormonal changes, but it cannot be considered the direct cause. It is difficult to explain a unilateral gynecomastia on the basis of trauma caused by

straps or packs which produce equal pressure on both sides of the chest.

The breast lesions reported by Lewis and North (30) among American prisoners of war are of interest in view of the obvious avitaminoses which were present at the time. The avitaminoses could have a direct bearing or could have acted indirectly by disturbing the endocrine production in the individual.

An hereditary factor is discredited in most instances but may be responsible for the condition known as pseudo-gynecomastia or enlargement of the breast area by adipose tissue without increase in actual glandular tissue. (28).

On the basis of endocrine etiology the condition may be arbitrarily divided into two groups: primary gynecomastia and secondary gynecomastia. In the primary group would fall those types in which a primary hormonal deficiency exists. In the secondary group would fall those types where there is an associated pathology with a primary natural or artificial increase of one or more of the body hormones.

The primary group would include the following: 1) spontaneous gynecomastia of puberty (13); 2) idiopathic gynecomastia without other physical signs of endocrine disturbance (6); 3) cases which develop postoperatively following prostatectomy (13); 4) cases associated with testicular atrophy secondary to disease or trauma (13); 5) a clinical type with endocrine dysfunction characterized by small testes, azoospermia, and an increased excretion of pituitary gonadotrophins.

The phenomenon of breast enlargement is frequently accompanied by defective genitalia, tendency to femininity and lack of sexual desire. Secondary characteristics such as hair growth may be normal. A case of this type was described by Brownstein (3) in 1939. In 1942 Klinefelter, Reifenstein, and Albright (20) described, with complete studies, nine cases of this type characterized by gynecomastia, aspermatogenesis without aleydigism and increased excretion of FSH (follicle-stimulating hormone). These authors felt that in the particular syndrome they described there was normal Leydig cell function and thus normal androgen production. Their studies showed atrophy of germinal epithelium in the testes with aspermatogenesis. The Leydig cells appeared normal or comparatively so in the cases biopsied. They felt the gynecomastia in their series was due to androgen acting on breast tissue in the absence of an X-hormone factor (called "inhibin" by McCullagh and Walsh), which is produced normally by the cells of the seminiferous tubules. They noted that there was a normal or slightly decreased excretion of the 17-ketosteroids. Their conclusion was that a factor of unknown origin caused a selective lesion of the seminiferous tubules resulting not only in aspermatogenesis but also in a lack of X-hormone (inhibin). They postulated that the lack of X-hormone (analo-

gous to estrin in the female), by failing to inhibit the FSH production in the pituitary, leads not only to the increase of FSH but, by failing to stimulate the luteinizing hormone production in the pituitary, to the decrease of LH as well. Consequently, the intact Leydig cells produce somewhat less androgen than normally. In the absence of X-hormone the androgen produced acts on the breast tissue during puberty to cause gynecomastia. As further evidence favoring their theory, they point out that male castrates rarely develop gynecomastia.

These authors did not feel that estrogen substances were in excess in their cases. Estrin levels were determined in two of their patients and found normal.

Heller and Nelson (15), investigating a group of 20 patients with the characteristic triad of aspermatogenesis, increased FSH excretion in the urine and small testes in which biopsy revealed hyalinization of tubules and clumping of the Leydig cells, concluded among other facts that gynecomastia was a variable sign associated with the basic triad. Fourteen of these cases exhibited gynecomastia. In the patients with this breast enlargement the urinary excretion of 17-ketosteroids, representing the androgenic substances of both the adrenals and testicles, was a low normal or subnormal. Four of the five cases with a four plus gynecomastia (using one plus to four plus as an index of involvement) had a normal urinary estrogen excretion. Of the remaining ten cases, the urinary estrogens were low in seven and not determined in three. There was a definite demonstrable rise in the urinary excretion of the pituitary gonadotropins in all cases. Four patients in their series with the most marked gynecomastia were remarkable, because, other than breast enlargement, there was only small evidence of disturbed endocrine dysfunction. These four patients with marked gynecomastia had normal hair distribution, voice pitch, muscular strength, phallus proportions and skeletal development.

These authors in a companion article (16) cited cases of gynecomastia occurring in prepuberal castrates. They felt that some member or precursor of the 17-ketosteroids complex might have mammogenic activity. This substance, the nature of which is admittedly unknown, is produced in the adrenal cortex and appears only, or occurs in increased amounts, under circumstances which have their origin in the failing function of the Leydig cells, with subsequent compensatory activity of the adrenal cortex. They also felt that there was no relation between the levels of excretion of estrogen and the condition of the breasts. However, they disagree with Klinefelter et al. (20), that the testes secrete a non-androgenic hormone, inhibin, which in addition to its action in inhibiting the production of the FSH factor by the hypophysis also inhibits the mammogenic action of testosterone. They felt that there was little evidence for the existence of

inhibin and for a mammogenic activity of testosterone.

The group of secondary gynecomastia, where there is a primary, natural or artificial increase of one of the body hormones, includes the following: (1) After administration of estrogens in carcinoma of the prostate which frequently results in unilateral or bilateral breast hypertrophy (7). (2) Gynecomastia produced, as reported by McCullagh and Rossmiller (21), following testosterone therapy. Pratt (23) reported a case of a typical 32 year old male eunuchoid who was given 150 mg. of methyl testosterone orally. Twenty-four hours later the mammary tree was three centimeters in diameter and the nipples were prominent. (3) Testicular neoplasms, such as teratoma, chorionepithelioma and carcinoma (13, 17). (4) Tumors of the adrenal cortex (10). Several cases have been reported where individuals treated with adrenal cortical extract have developed enlarged breasts. It is believed that excess adrenal cortex secretion suppresses testicular androgen production (3). (5) Part of a syndrome associated with pseudohermaphroditism and a true hermaphroditism (13). (6) Hepatic cirrhosis (9). It is believed the liver normally plays a role in the inactivation or destruction of estrogen. Most writers mention that atrophy of the testes has been present in such cases (9). An explanation of the absence of gynecomastia in acute hepatic necrosis and in the majority of cases of cirrhosis is not yet apparent. (7) Pineal and pituitary tumors (13). (8) Anterior pituitary disturbances (13). (9) Hyperthyroidism (13). (10) Cases of bronchogenic carcinoma in which gynecomastia was present have been reported. In one of the cases studied, normal androgen values were found in the urine with an increase of estrogens (8).

Taylor (27), reviewing the subject of chronic cystic mastitis in the female, which on occasion has a microscopic appearance (26) similar to gynecomastia, failed to reach a definite conclusion regarding the etiology of chronic cystic mastitis. There is some evidence (11) that an endocrine disturbance is the cause of chronic cystic mastitis in the female. It is possible that evidence regarding the cause of the condition in one of the sexes will assist in understanding the similar disturbance in the opposite sex. A theoretical consideration in explanation of the higher incidence of chronic cystic mastitis in the female may be the fact that less disturbance of the hormonal balance is required in the female (as compared to the male) to cause the female's endocrine status to approach the A:E ratio found in gynecomastia. Furthermore, the great monthly cyclic variation of the female's endocrine balance is not present in the male.

When considering the endocrine etiology one must bear in mind the close structural relationship of the estrogens and androgens. The possible conversion of androgen to estrogen or vice versa within the body should not be forgotten (2).

DIFFERENTIAL DIAGNOSIS

In the differential diagnosis of gynecomastia, three breast conditions one should consider are: 1), infectious processes, 2), increase in the subcutaneous tissue of the breast, and 3), neoplasms (11).

The infectious processes include those of pyogenic, luetic and tuberculous origins.

Geschickter (11) points out that 77 per cent of boys between the ages of 13 and 16 years manifest a button-shaped subareolar node of palpable size. It is the result of hyperplasia of duct epithelium and periductal stroma. The node tends to disappear at about the age of 17 years. Geschickter (11) states it is incorrect to call this node gynecomastia.

The term pseudogynecomastia has been applied to the condition where there is an increase of fat in the mammary region associated with obesity of feminine distribution.

Few of the neoplastic lesions present a clinical picture similar to gynecomastia. These lesions are fibromas, adenomas, lipomas, hemangiomas, cysts, and papillomas. Occasionally sarcomas and carcinomas present a similar picture.

PATHOLOGY

In Geschickter's (11) series of 108 cases 86 per cent were unilateral. In a review of 71 cases operated on at Brooke General Hospital (31), three cases had bilateral involvement and the remainder were unilateral. The distribution between right and left mammary gland involvement in the latter was equal. It was noted that many of these cases grossly presented a single nodule, but that microscopically they were reported as gynecomastia.

According to Geschickter (11) there are three gross types: 1) the diffuse hypertrophic type seen in early puberty (in such cases the enlargement is usually moderate), 2) the fibro-adenomatous type which is more definitely nodular, and 3), the true feminization of the breast where the size of one or both glands approach that seen in the adult female.

Microscopically, the breast shows "a growth of mammary ducts and periductal stroma. Lobule formation is absent. There is hyperplasia of the duct epithelium with moderate desquamation of cells into the lumen. Small papillary-like projections may form in the lining of the ducts and occasionally the ducts are dilated and contain secretion. A moderate periductal infiltration of wandering cells occurs. There may be an accompanying hypertrophy of the subcutaneous sweat glands. The entire picture may be indistinguishable from the early development of the normal female breast at puberty" (11). In the nodular type almost pure fibrous tissue may be found (11). In some cases (26) the changes are identical with those seen in chronic cystic mastitis in the female.

THERAPY

The primary etiology should be treated whenever possible. There is also need for treatment of the enlarged gland because of the psychic embarrassment, especially under conditions where large groups of men are living together in total lack of privacy.

"The treatment of gynecomastia resolves itself into two divergent schools of thought: those who believe the great majority of cases will respond to androgens, especially if the condition is bilateral, and those who believe surgery is the method of choice" (26).

Adair (1) feels that unless the condition responds to testosterone it should be considered mastitis rather than gynecomastia, and states further that the best treatment for the condition is hot compresses and scientific neglect. Bronstein and Cassorla (4) believe hormonal therapy with androgenic substances is not indicated where sexual development is adequate. Hoffman, according to Geschickter (11) reported favorable results with 25 milligrams of testosterone intramuscularly two times weekly for ten to twelve weeks. McCullagh and Rossmiller (21), however, have reported the production of breast enlargement following testosterone therapy.

For cosmetic reasons surgery is the treatment of choice, but should be postponed until it is evident that the enlargement will not regress spontaneously (11). Gooel (14) recommends a slightly curved, transverse incision, inferior to the areola, which allows for the excision of the entire breast and offers approach to all positions including the axilla.

PRESENTATION OF CASES

Case 1: A twenty-year-old, unmarried white male had noticed a slight increase in the size of his left breast during February of 1945. There had been a gradual increase in the size of the gland until February 1946 at which time he presented himself at our dispensary. At no time was there any tenderness of the breast, reduction in size or secretion. When he first noticed the enlargement, the soldier thought he had possibly incurred some trauma to the left breast, but was not certain. He had found it unnecessary to shave until eighteen years of age. At present he does not shave more than twice a week. Erection, ejaculation and intercourse had been satisfactory, but a decided lack of interest in the opposite sex was related. Prior to entering the Army he was employed as a farm laborer. After taking 15 weeks of basic infantry training, he was assigned as a vehicle driver which was his assignment when seen by us.

There was no history of jaundice, mumps, orchitis or trauma to the testicles.

After careful studies the involved gland was removed surgically.

Case 2: A twenty-year-old, unmarried white male reported on sick call with an enlarged left mammary gland. He had been examined for the above complaint three months earlier, but the enlargement had been questionable at that time. Since then there had been a gradual increase of the breast tissue to the presenting size. At no time was a reduction in size of the involved gland, or a secretion from it noted. Occasionally some

tenderness on pressure had been present. No history of trauma to the breast could be elicited. The history of past illnesses revealed mumps at the age of four with no associated orchitis. No injury to the testicles was remembered and the patient did not believe his testes were ever larger than at the time when first examined by us.

He began to shave at the age of seventeen and at present shaves daily. Erection and ejaculation were reported as having been satisfactory, but intercourse had not been attempted. A greater lack of interest in the opposite sex existed than in Case 1.

For six months after completing high school he was employed as a manual laborer. After taking 21 weeks of basic infantry training, he was assigned as an infantryman for 14 months. During four months prior to the enlargement of his breast he had been assigned as a clerk.

The involved gland was removed surgically after careful studies.

The pertinent physical findings, and laboratory results in the two cases are tabulated in Tables I and II, respectively.

Perirenal air injections (24) for roentgenographic studies were not performed in view of the lack of reason to suspect active adrenal gland pathology and the occasional mortality associated with the procedure.

TABLE I. PHYSICAL EXAMINATION

	Case No. 1	Case No. 2
Age	20	20
Weight	170 pounds	198 pounds
Blood pressure	115/80	140/90
Duration of Gynecomastia	1 year	4 months
Degree of Gynecomastia	Advanced	Moderate
Height	65½ inches	72½ inches
Span	67 inches	72 inches
Body length, pubis to crown	33½ inches	34¼ inches
Width of shoulders	17 inches	16½ inches
Width of hip, iliac crest	11½ inches	12½ inches
Width of hip, Fem. trochanter	13 inches	14½ inches
Muscular development	Normal	Normal
Prostate size	½ average	Average
Penis	Normal	Normal
Testes, average size (in cm.)*	5×2.5×3.75	2.5×1.25×1.25
Hair distribution		
Head hair	Heavy	Heavy
Hairline withdrawal	None	None
Facial hair	Sparse	Normal
Axillary hair	Normal	Normal
Body hair	Absent	Normal
Pubic hair	Male	Male
Extremity hair	Absent	Absent

* Average testicular measurements (4.5 cm. in length, 2.5 cm. in breadth and 3 cm. in the anterior-posterior diameter) (From Gray, Henry, edited by Lewis, Warren H.. *Anatomy of the Human Body*, Ed. 24, Philadelphia, Lea and Febiger, 1942, page 1260.)

TABLE II. LABORATORY EXAMINATIONS

	Case No. 1	Case No. 2
Roentgenographic studies		
Skull, abdomen, chest	Normal	Normal
Sella tursica	Normal	Normal
Bone age	Normal	Normal
Excretory urogram	Normal	Normal
Blood studies		
R.B.C., W.B.C., HB., diff.	Normal	Normal
Hematocrit	40% V.P.C.	40% V.P.C.
Sedimentation rate (60 min.)	7 mm.	14 mm.
Blood chemistry		
Urea N. mg. % (whole blood)	4	10
Total blood protein Gms./100 cc.	7.7	7.5
Serum albumin Gms./100 cc.	5.2	5.3
Serum globulin Gms./100 cc.	2.5	2.2
Serum cholesterol	182	174
Cephalin flocculation test	Two plus	Negative
Hippuric acid test (intravenous)	1.0 gm.	1.2 gm.
Icteric index	2.0	2.5
Kahn	Negative	Negative
Glucose tol. (Rose-Exton)	Normal	Normal
Insulin tolerance	Normal	Decreased
Basal metabolic rate	Minus 5%	Minus 7%
Urine examination		
Routine and micro.	Negative	Negative
Bile salts and pigments	Negative	Negative
Urobilinogens mgm. %	1.9	1.7
FSH mouse units/24 hours	Increased	Increased
17-ketosteroids	Normal	Decreased
Estrogens-urinary-mouse units/24 hrs.	Not increased	Not increased
Spermatic fluid examination		
Volume	4.2 cc.	5.0 cc.
Sperm count/cc.—Average	30,000,000	Azospermia
Spermatozoa		
Motility	50% amotile	—
Morphology	Normal	—
Microscopic diagnosis—breast	Gynecomastia	Gynecomastia

DISCUSSION OF CASES

Both cases had diffuse enlargement of the mammary gland resembling the female breast at puberty, which is considered to be representative of true gynecomastia. The microscopic picture of the breast was typical in each case.

In Case 1 the only significant finding beside the breast enlargement was

the persistent oligospermia averaging 30,000,000 spermatozoa per cubic centimeter. The two plus cholesterol-cephalin flocculation test could not be explained. In the absence of any other marked deviation from normal in either physical or laboratory examinations, it was difficult to make a specific conclusion as to the etiology of the gynecomastia in this case. The soldier's sparse beard, admitted absence of libido and deficient spermatogenesis indicated a possible endocrine factor; although the 17-ketosteroids,

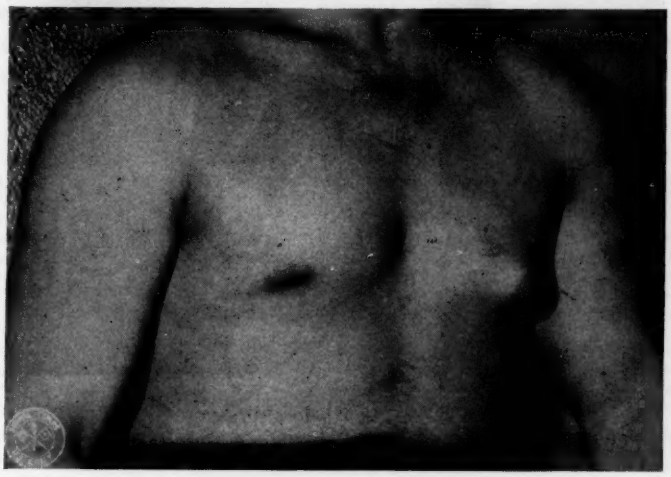

FIG. 1. *Case 1* before surgery.
(Photo by U. S. Army Signal Corps.)

and estrogen urinary excretion were within normal limits, the FSH excretion was considerably elevated.

We felt this case might be in an early stage of the syndrome first described by Klinefelter's (20) group and later by Heller and Nelson (15). Another consideration was a delayed maturation of the patient from a prolonged puberal stage, in which case the breast enlargement would fall into the group of spontaneous gynecomastia of puberty, which had failed to recede. A less satisfactory third classification would be to label the case as an idiopathic gynecomastia.

Case 2 was a eunuchoid type individual evidenced by the disproportion of body length (29), 34¼ inches (crown to pubis), to the lower extremity length, 38¼ inches (pubis to sole), the subcutaneous fat distribution and atrophic testes. He was classed as a functional prepubertal castrate by us. The history of mumps may have significance as regards the atrophic testes.

However, as mentioned in the case presentation he did not remember any involvement of his testes, had no history of trauma to his testes and no memory of his testes being larger than when examined by us. His markedly atrophic testicles along with the azoospermia, increased FSH excretion,

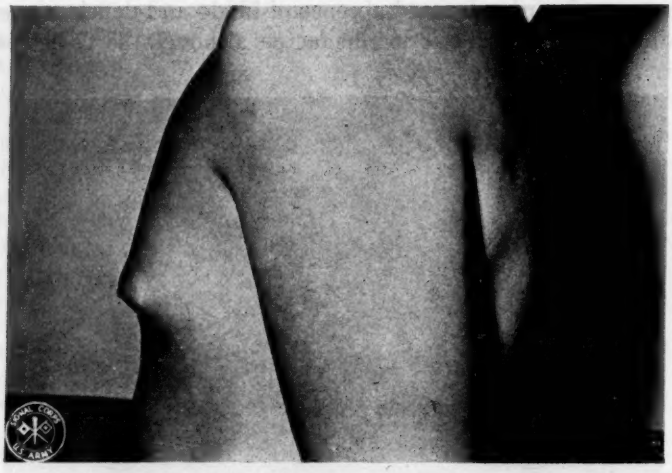

FIG. 2. *Case 2* before surgery.
(Photo by U. S. Army Signal Corps.)

decreased 17-ketosteroids excretion and normal estrogen excretion caused us to consider this case as belonging to the group of cases described by both Klinefelter et al. (20) and Heller and Nelson (15).

SPECIAL LABORATORY PROCEDURE

The urinary gonadotropins were determined in the manner described by Klinefelter, Albright and Griswold (19). This procedure involves the use of 21-day-old virgin female white mice weighing not more than 6-10 grams when sacrificed—preferably less than 9 grams. The gonadotropins in the urine were extracted and concentrated by the non-dialysis method described by the above mentioned authors. A total quantity of 2.5 cubic centimeters was injected into each mouse in appropriate dilutions. A uterine weight of 7 milligrams or above after the uterus was blotted dry was the criterion for establishing a positive mouse test. However, primary changes in the ovaries and secondary changes of the uterine tubes were also examined for evidence of gonadotropic action. To avoid possible error

in calculating the number of gonadotropic mouse units excreted in 24 hours, the entire 24-hour urine excretion was collected, mixed thoroughly and a 90-minute aliquot used for the tests. An attempt was made to standardize the "blotting dry" of the uterus by placing the uterus between two pieces of filter paper and allowing a 100 gram weight to rest on the uterus for 60 seconds.

The test apparently is chiefly a test of the FSH (follicle stimulating hormone) in the urine. The daily male excretion of FSH is not less than 6.6 mouse units and not more than 96 mouse units per 24 hours (19). The results of tests in the cases presented in this paper and the controls are expressed in Table III.

TABLE III. GONADOTROPINS

Case No.	Av. Wt. of Mice at Sacrifice (Gm.)	Average Uterine Weight of mice (mg.)				Uterine Tubes (engorgement and edema)				Ovaries of Mice (Evidence of follicle maturation)			
		No urine inject	For 96 u. per 24 hr.	For 144 u. per 24 hr.	For 192 u. per 24 hr.	No urine inject	For 96 u. per 24 hr.	For 144 u. per 24 hr.	For 192 u. per 24 hr.	No urine inject	For 96 u. per 24 hr.	For 144 u. per 24 hr.	For 192 u. per 24 hr.
Control	7.8	8.84	5.76	—	—	Neg.	Neg.	—	—	Neg.	Neg.	—	—
1	7.1	—	7.2	8.8	7.9	—	Neg.	Neg.	Neg.	—	Neg.	Neg.	Neg.
2	7.4	—	7.6	6.0	6.3	—	Pos.	Pos.	Neg.	—	Neg.	Neg.	Neg.

Note: According to Klinefelter, Albright and Griswold (19), the upper limit of normal male FSH (Follicle Stimulating Hormone) excretion is 96 units per 24 hours.

It was noted that mice receiving no urine injections had uterine weights consistently above the uterine weights of those mice which received injections of urine from the individuals who were apparently normal endocrinologically. These concentrates were prepared by the nondialysis method (19), used for all our FSH tests. No mice used in the tests died from the urine injections. As in all biological tests, one or two mice are not a reliable index for any given reading.

The urinary estrogens were determined on 21-day-old female white mice using aqueous solutions made from the ether precipitates of aliquots of total 24-hour urine specimen. The uterine weight increase in the mice, as used by Scott and Vermeulen (25), was the criterion for evidence of estrogenic action. Uterine weights above 7 milligrams were considered to be a positive mouse test. The uterine tubes were also examined for evidence of estrogenic action. These findings were compared to similar determinations made from aliquots of normal male 24-hour urine specimens. The results of these tests are expressed in Table IV.

The 17-ketosteroids were determined on a portion of a 24-hour urine

TABLE IV. ESTROGENS

Case No.	Av. Wt. of Mice at Sacrifice (Gm.)	Average Uterine Weight of mice (mg.)				Ovaries of Mice (Evidence of follicle maturation)			
		For 8 u. per 24 hr.	For 16 u. per 24 hr.	For 64 u. per 24 hr.	For 8 u. per 24 hr.	For 16 u. per 24 hr.	For 16 u. per 24 hr.	For 32 u. per 24 hr.	For 64 u. per 24 hr.
Control	6.6	6.6	5.1	5.7	5.9	6.9	Neg.	Neg.	Neg.
1	7.0	11.6	7.8	5.4	5.3	Neg.	Pos.	Neg.	Neg.
2	6.5	—	12.9	6.6	—	—	Neg.	Neg.	—

specimen by the Pincus colorimetric assay* as described by Cahen and Salter (5). The normal values for the 17-ketosteroids given for the method are 8 to 23 milligram-equivalents of androsterone per 24 hours. The results are shown in Table V.

TABLE V. 17-KETOSTEROIDS

	Normal Values	Case No. 1	Case No. 2
17-Ketosteroids excretion (mg. per 24 hr.)	8–23	12.6	5.9

The 17-Ketosteroids are expressed in terms of milligram-equivalents of androsterone.

COMMENT

The numerous theories put forth concerning the etiology of male breast enlargement are difficult to evaluate in view of the present comparatively limited knowledge of the endocrine functions occurring in the body. That trauma can be a factor in some cases must be admitted. The "lack of inhibin" theory of Klinefelter et al. (20) is worth considering and not without basis. Heller and Nelson's (15) proposal of an abnormal mammogenic 17-ketosteroid being the cause of gynecomastia requires further experimental evidence. In our opinion the altered A:E ratio is a constant factor in the cases reviewed. The fact that the 17-ketosteroids urinary excretion was consistently low or sub-normal in most of the cases of Klinefelter et al. (20) and also those of Heller and Nelson (15) lends further credulence to this conception. It is also to be noted that the spontaneous gyneco-

* The androsterone for the 17-ketosteroid colorimetric assay was supplied through the courtesy of the Ciba Company, Summit, New Jersey.

mastia of puberty is associated with a period of radical alteration of the production of androgens and estrogens in the body.

One of the most constant factors encountered by Klinefelter et al. and Heller and Nelson was the persistent elevation of gonadotropins. This increase is believed to be the result of the removal of the normal inhibiting influence of the testicular hormone, testosterone. The gonadotropins are also believed to increase at the time when the spontaneous gynecomastia of puberty occurs. A possible mammary gland stimulating action of the gonadotropins in the presence of androgen may be considered.

A final observation on the phenomenon is that while functional prepubertal castrates develop gynecomastia occasionally, complete surgical castrates apparently do so rarely. The majority of patients with gynecomastia evidently show varying degrees of reduction of testicular function. Clinical observations (21, 23) tend to substantiate the idea that a certain level of androgenic substance must be present in the body before gynecomastia can develop.

SUMMARY

Two cases of gynecomastia were presented with laboratory data, and the following points were discussed: (A) Incidence; (B) Etiological factors; (C) The endocrine disturbances possibly responsible for the condition; (D) The pathology and occasional similarity of the microscopic picture of gynecomastia to that of chronic cystic mastitis in the female; (E) The necessity for treatment of gynecomastia because of the mechanical irritation and psychic embarrassment; (F) The hormonal therapy and surgical removal of the involved breast in gynecomastia.

CONCLUSION

Gynecomastia is the result of a disturbed endocrine status usually related to an alteration of the normal androgen:estrogen ratio within the individual. In those patients where no cause for the phenomenon can be ascertained, our failure is probably due to the inadequacy or incompleteness of present methods of examination. If possible the primary cause should be treated; when impossible, the present therapy of choice is surgical removal of the enlarged gland.

ACKNOWLEDGEMENT

We gratefully acknowledge the cheerful assistance of Miss Addelia Peterson and Major William F. Hettler of the United States Army Sanitary Corps in performing the special laboratory tests required for our studies. We also wish to express our appreciation for the use of the facilities of the United States Army Eighth Service Command laboratory, Fort Sam Houston, San Antonio, Texas.

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31. From pathology reports, Brooke General Hospital Laboratory, Fort Sam Houston, Texas.
32. From Surgical Service, Brooke General Hospital, Fort Sam Houston, Texas.

ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 P. M. and will be preceded by a cocktail party, the location of which will be announced later. The Council will meet at 2 P. M. Thursday, June 17th.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

Announcement of Award

AMERICAN ASSOCIATION FOR THE STUDY OF GOITER

VAN METER PRIZE AWARD

The American Association for the Study of Goiter again offers the Van Meter Prize Award of Three Hundred Dollars and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Toronto, Canada, May 6th, 7th, 8th, 1948 providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy sent to the corresponding secretary, Dr. T. C. Davison, 207 Doctors Building, Atlanta 3, Georgia not later than February 1st, 1948. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association. This will not prevent its further publication, however, in any Journal selected by the author.

T. C. DAVISON,
Corresponding Secretary

OBITUARY

Ernst Laqueur, 68, Professor of Pharmacology at the University of Amsterdam, Holland, died on August 19, 1947, while vacationing in Switzerland.

He was the discoverer of testosterone which name he coined. He also did fundamental work on the standardization of insulin and estrogens and belonged to the Committee of the League of Nations which established the International Standards of estrone and estradiol benzoate. He inaugurated the clinical use of androgens in prostatic hypertrophy and treated Cushing's syndrome successfully with massive doses of estradiol benzoate.

In 1946 he was awarded the Berzelius Medal by the Swedish Medical Society.

Doctor Laqueur founded the Acta Brevia Neerlandica and was one of the originators of the Excerpta Medica, the publication of which is just starting.

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor; D. A. McGINTY. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

ADRENALS

THELANDER, H. E. Congenital adrenal-cortical insufficiency associated with macrogenitosomia. *J. Pediat. 29:* 213 (1946).

The author reports the interim story and final study of a case of congenital adrenal-cortical insufficiency associated with macrogenitosomia which he had reported previously (*J. Pediat. 18:* 779 (1941)). This boy was studied for six years to the time of his death with measles. The complete case history and findings at post-mortem examination are given. A table is provided of weights and measurements taken at about yearly intervals. It was estimated that, if the boy had lived, his ultimate height would have been about 5 feet, in other words below average. His teething corresponded with his chronologic but not with his physiologic age. The facial contour, voice, appearance, and distribution of body hair were that of an adolescent; the testicles were small and undeveloped. Mentally and emotionally the child did very well. At various times the patient received whole adrenocortical extract, adrenocorticotropic hormone, and particularly desoxycorticosterone acetate. This latter medication was administered sub-lingually in a dosage of ½ mg. (2 drops) four times a day. This dose was subsequently increased to five times daily, and finally to 4 drops five or six times a day. When the dosage was reduced, he was restless and irritable, had a poor appetite, and drank excessive amounts of water; however, he had no craving for salt. The presence of androgenic hormones was best illustrated in the growth of the child who was husky and energetic. At post-mortem he was found to have bilateral adreno-cortical hyperplasia which was extreme, and he had aberrant adrenocortical rests in both testes. He also had a persistent thymus, but aside from this the organs were normal. In spite of the extreme rapidity of his growth, the striated muscle of the body and the muscles of the heart were normal, and the bony structure, teeth and organs were well formed. There is no evidence of lack of mineralization or avitaminosis. There was a family history of disease involving the adrenal cortex, since a sibling who died with somewhat similar symptoms, probably had hypospadias and was presumably a female pseudohermaphrodite.—*E.C.R., Jr.*

TESTES

DELORY, G. E. Seminal fluid acid phosphatase in sterility. *Brit. M. J. 1:* 566 (1947).

Samples of seminal fluid from 36 men being investigated for sterility were examined

for acid phosphatase, pH, morphology, motility, and abundance of spermatozoa. No correlation was found between the enzyme concentration and either the number of spermatozoa or their motility. There was no evidence that the acid phosphatase is consistently low in male sterility.—*L.T.S.*

MacLeod, J. and R. S. Hotchkiss. Semen analysis in 1,500 cases of sterile marriages. *Am. J. Obst. and Gynec. 52* (1): 34–41 (1946).

Deficiencies either in spermatozoa count, motility or morphology, were found in about 50 per cent of 1,500 patients who had semen analysis because of presumed sterility. The authors found that as the spermatozoa count fell below 60 million per cubic centimeter other defects, such as motility and abnormal morphology, became more apparent, until in the very low count range all three deficiencies together are likely to be found. It is suggested that the 60 million per cubic centimeter spermatozoa count level is a reasonably dividing line between good and impaired fertility.—*C.D.D.*

OVARIES

Ayre, J. E. and W. R. Foote Granulosa-cell tumor with pregnancy following removal. *Am. J. Obst. and Gynec. 51* (2): 260–264 (1946).

The authors report a granulosa-cell tumor of the ovary in a 35-year old woman who had had amenorrhea for four years prior to operation. Menstruation returned promptly following operation. Fertilization occurred, and the resulting pregnancy carried on normally to maturity.—*C.D.D.*

Bianco, J. J. and G. O. Favorite. Granulosa-cell tumor of the ovary. *Am. J. Obst. and Gynec. 52* (4): 677–680 (1946).

A case report is presented of a granulosa cell tumor occurring in a 55-year old woman. —*C.D.D.*

Chesley, J. C., S. A. Cosgrove and J. Preece. Hydatidiform mole, with special reference to recurrence and associated eclampsia. *Am. J. Obst. and Gynec. 52* (3): 311–320 (1946).

The authors found 40 instances of recurrent hydatidiform moles and only 5 of these women had normal pregnancies occurring between moles. Thirty-five instances of probable or alleged eclampsia in conjunction with hydatidiform mole were encountered. In over 75,000 deliveries, 57 hydatidiform moles were found, an incidence of one in 1,321 deliveries. Three of these moles were followed by chorionepithelioma.—*C.D.D.*

Curtis, A. H. The origin of adrenal-like tumor of the ovary. *Am. J. Obst. and Gynec. 52* (1): 115–122 (1946).

A case report is presented of an adrenal-like tumor of the ovary in a 23 year old girl. This is the sixteenth such case to be reported in the literature. The author demonstrates in photomicrographs a gradual transition from a polygonal adrenal type of cell to a stroma composed of spindle-shaped cells. The author believes that study of this tumor makes it apparent that adrenal-like tumors of the ovary arise by metaplasia of stroma cells or other indifferent cells, rather than from embryologically misplaced tissue in the ovary.—*C.D.D.*

CURTIS, A. H. Another case of arrhenoblastoma. *Am. J. Obst. and Gynec. 52* (1): 128–131 (1946).

The patient was a 52 year old woman who had had signs and symptoms of masculinization for six months prior to surgery. No hormonal assays are reported. Postoperatively the voice modulated, the hypertrichosis largely disappeared and the clitoris returned to normal size. The length of the followup period is not stated.—*C.D.D.*

FARBER, E. P. The induction of labor with methergine: preliminary report. *Am. J. Obst and Gynec. 51* (6): 859–865 (1946).

A new synthetic ergot preparation was employed in the medical induction of 43 consecutive patients, and satisfactory results were obtained in 39 instances. The reported dosage was 0.5 cubic centimeter given orally in about an ounce of water and then 1 cubic centimeter every 30 minutes for 4 doses. There were no untoward effects noted on mother or fetus.—*C.D.D.*

FARRIS, E. J. A test for determining the time of ovulation and conception in women. *Am. J. Obst. and Gynec. 52* (1): 14–27 (1946).

Observations were made on 100 women, 12 unmarried and 88 married. Two cubic centimeters of a voided morning specimen of urine was injected subcutaneously into 2 immature Wistar rats ranging between 22 and 25 days of age, and weighing between 30 and 50 grams. The animals were sacrificed in 2 hours and the degree of redness of the ovaries was compared with the colors of the Munsell color chart. The normal positive reaction consists of a definite deepening of the ovary of the rat for 3 or 4 consecutive days near the middle of the menstrual cycle. The abnormal reactions include no color, one or two days only of color, or a split reaction—one or two days of color separated by no color for one, two or more days. Urine assays showed gonadotropin present when the hyperemic reaction was positive. The author believes that, on the basis of artificial insemination studies, conception is most apt to occur on the third or fourth day of a normal reaction.—*C.D.D.*

FOOTE, E. C. AND C. E. SEEGAR JONES. An evaluation of the Hogben pregnancy test. *Am. J. Obst. and Gynec. 51* (5): 672–677 (1946).

An analysis of 157 Hogben pregnancy tests is reported in the following conditions: 60 intrauterine pregnancies, 11 abortions, 2 ectopic pregnancies, 1 abdominal pregnancy and 68 nonpregnant patients with miscellaneous diagnoses. No false positive tests were obtained. The test is apparently not accurate before the fortieth day of gestation. The authors have a 95 per cent accuracy with this test and believe it compares favorably with the Friedman test in accuracy.—*C.D.D.*

GOLDSTINE, M. T. Arrhenoblastoma of the ovary: report of two cases. *Am. J. Obst. and Gynec. 52* (1): 123–127 (1946).

The author presents brief case reports of two young patients, 25 and 20 years of age, in whom signs and symptoms of masculinization led to the surgical removal of arrhenoblastomas. Both had uneventful recoveries. Both had return of normal ovarian function. One delivered a full term normal infant 19 months after surgery and the other was at the time of this report in her thirty-second week of a normal pregnancy 14 months following surgery.—*C.D.D.*

HAMBLEN, E. C. Some contributions of endocrinology to obstetrics and gynecology. *Am. J. Obst. and Gynec. 51* (6): 796–803 (1946).

A few of the significant endocrine contributions to obstetric and gynecologic thought and practice have been surveyed. It is not too much to hope that the next fifty years may bring many further endocrine solutions to physiologic, diagnostic and therapeutic problems of our specialty. It may well be that the ultimate solution of eclamptogenic toxemia and genital carcinoma may be effected through the medium of an expanding knowledge of the endocrine system (Author's Summary).—*C.D.D.*

HARDING, F. E. The oral use of hexestrol for estrogen deficiency. *Am. J. Obst. and Gynec. 51* (5): 660–665 (1946).

Ninety patients were treated with oral hexestrol in average daily doses of 1 to 2 milligrams. Thirty-two of these were in the climacteric group, but 16 were still menstruating, 3 regularly. Twenty-five had artificial menopause, but 6 of these had only subtotal hysterectomies. The remaining 33 patients had other conditions with a suspected ovarian deficiency. The author reports that all 90 patients were treated with hexestrol with satisfactory results. A low incidence of toxic reactions (3 to 6 per cent) is reported. It is reported that there were less undesirable effects upon uterine bleeding with hexestrol than with conjugated estrogens-equine, diethylstilbestrol propionate and ethinyl estradiol.—*C.D.D.*

HAUCK, H. M. Plasma levels and urinary excretion of ascorbic acid in women during the menstrual cycle. *J. Nutrition, 33* (5): 511–515 (1947).

The author studied the plasma levels and urinary excretion of ascorbic acid relative to the menstrual cycle. The subjects were 10 women who were maintained on constant amounts of ascorbic acid for periods of 4–6 weeks. No unusual variations in the plasma levels or excretion of the vitamin were found when mid-cycle values were compared with those obtained on the first day of menses.—*H.S.G.*

JONES, G. S. AND H. S. EVERETT. Arrhenoblastoma of the ovary, with a report of two cases. *Am. J. Obst. & Gynec. 52* (4): 614–622 (1946).

The authors report 2 patients with a pathological diagnosis of arrhenoblastoma. One had no bleeding for 8 months, but little else except considerable hirsutism to suggest masculinization. The other had definite masculinization including hoarseness and enlargement of the clitoris. In the latter patient, preoperatively the urinary 17-ketosteroids were 56 and 36 milligrams per 24 hours on two separate occasions, and 12 days postoperatively the level had fallen to 11 milligrams per 24 hours. No pregnanediol was present in the urine. This tumor was composed almost entirely of interstitial cell elements with only occasional tubular formation.—*C.D.D.*

LUBIN, S. The routine use of stilbesterol for engorgement and lactation in nonnursing mothers. *Am. J. Obst. and Gynec. 51* (2): 225–229 (1946).

One hundred postpartum patients who did not nurse their babies were studied as follows: One-half the group served as controls. The other half were given oral diethylstilbesterol, 10 milligrams, three times daily for two days, followed by 5 milligrams three times daily until the patient was discharged from the hospital. This regime was started

within the first 24 hours after delivery, and patients were observed for pain, engorgement, milk, fever and erythema of the breast. Pain was absent in 58 per cent of the control group and 88 per cent of the treated group. Milk secretion was affected as follows: control group—leakage, 76 per cent and expressed milk, 24 per cent; diethylstilbesterol group—leakage, 34 per cent and expressed milk, 56 per cent. The author points out that 50 per cent of the treated group developed full, painful breasts at some time within two weeks after leaving the hospital. He concludes that while the oral administration of diethylstilbesterol will prevent or postpone engorgement of the breast and its attendant pain, the advisability of its routine use in nonnursing mothers is questionable.—*C.D.D.*

LYON, R. Pregnanediol excretion at the onset of labor *Am. J. Obst. and Gynec. 51* (3): 403–410, (1946).

The author studied sodium pregnanediol glycuronidate levels in the late antepartum and postpartum periods of premature, normal and postmature deliveries. The titrimetric method of Allen and Viergiver, with a modification of extraction technique suggested by Woolf, Viergiver and Allen, was employed. The composite of 68 assays, in patients who delivered spontaneously, showed the following levels: 5 days antepartum, 30 mg; 2 days, 19.5; 1 day, 14.8; the day of delivery 12.5; first day postpartum, 8.8 and second day 4.6 mg. of sodium pregnanediol glycuronidate per 24 hours. There were no marked differences in values whether the delivery occurred prematurely, normally, postmaturely or by cesarean section. The author believes his data implies that the concentration of progesterone available at the onset of labor is insufficient to maintain and continue pregnancy.—*C.D.D.*

MCCORMACK, G. A comparison of the color chemical test with the Friedman modification of the Aschheim-Zondek test. *Am. J. Obst. and Gynec. 51* (5): 722–725 (1946).

The chemical color reaction test for pregnanediol was done simultaneously with the Friedman test on 304 patients. Of this number, 262 were in agreement. Negative chemical tests and positive Friedman tests were obtained on 38 patients diagnosed as threatened abortion. Two instances of hydatid mole were tested. In both instances, the chemical tests were negative and the Friedman positive. Corpus luteum cysts were apparently encountered twice with the anticipated results in assay being obtained. The author believes the two tests have an equivalent degree of accuracy and points out that their combined use can be helpful in establishing certain diagnoses.—*C.D.D.*

MENDEL E. B. Chiari-Frommel Syndrome: a historical review with case report. *Am. J. Obst. and Gynec. 51* (6): 889–892 (1946).

A case report is presented. One and one-half years after the birth of the baby, uterine bleeding returned more or less regularly, but intermittent secretion of watery fluid from breasts continued for 2 years. No further pregnancies occurred but a secretory endometrium was found on one occasion.—*C.D.D.*

MORROW, A. G. AND R. S. BENUA. An evaluation of the Guterman pregnancy test. *Am. J. Obst. & Gynec. 51* (5): 685–691 (1946).

The authors modified Guterman's original technique by substituting 1 cubic centimeter of 20 per cent solution of sodium in 95 per cent ethanol for 10 cubic centimeters of 2 per cent sodium hydroxide in absolute methanol, and by substituting Number 42

Whatman filter paper instead of a fritted glass filter. With this altered technique, several falsely positive results were obtained during the luteal phase of the menstrual cycle.

The authors point out that the test is not reliable in the presence of functioning corpus luteum tumors and probably also in association with an arrhenoblastoma.—*C.D.D.*

RAKOFF, A. E. Studies on high dosage progesterone therapy of amenorrhea. *Am. J. Obst. and Gynec. 51* (4): 480–490 (1946).

The author studied 51 patients, 7 of whom had primary amenorrhea and 44 had secondary amenorrhea. The duration of the latter varied from 2 to 13 years in 19, 1 to 2 years in 5, 6 months to one year in 14 and less than 6 months in 6 patients. Initial therapy generally consisted of intramuscular injection of 20 milligrams of progesterone on each of three consecutive days. If no bleeding occurred, priming with small amounts of oral estrogens was employed and the series of progesterone injections repeated. None of those with primary amenorrhea had bleeding after progesterone alone. Induced bleeding occurred in 5 who were primed with estrogens and then were given the progesterone. Sixty-six per cent of those with secondary amenorrhea responded to progesterone alone. The remainder had bleeding following the dual therapy. The most interesting part of the report concerns 2 of the patients with primary amenorrhea. These were of short stature, had sexual infantilism and high gonadotropins. More and more instances of this syndrome are being recognized.—*C.D.D.*

ROSSMAN, I., AND G. W. BARTELMEZ. Delayed ovulation, a significant factor in the variability of the menstrual cycle. *Am. J. Obst. and Gynec. 52* (1): 28–33 (1946).

A review is presented of the variability in the length of the pre- and postovulatory phases of the menstrual cycle in the macaque. The author reports ovulation on the 21st to the 23d, 29th, 29th to the 30th, 30th and 36th days after the onset of normal menstrual periods in a colony of healthy mature macaques. Delayed ovulation in these instances was due to a postmenstrual period of ovarian inactivity. The author believes such delayed ovulation may be relatively frequent in occurrence.—*C.D.D.*

RUTHERFORD, R. N. Preconceptional progestin therapy in habitual abortion. *Am. J. Obst. and Gynec. 51* (5): 652–659 (1946).

A series of 63 patients, each of whom had had 3 or more successive abortions, were treated with 5 milligrams of progesterone intramuscularly every other day from the eighteenth day until the menstrual flow supervened. If the period was missed, the injections were continued as before until the beginning of the fourth month and then every third day until fetal motion was felt.

Of the 54 patients who had had 3 successive abortions, 31 delivered viable infants and 28 survived, a corrected percentage of 51.8. Of those who aborted, there were proved pathologic ova in 69.1 per cent. Of the 9 patients who had had 4 successive abortions, 4 carried pregnancies to viability and successful termination. Three of the 5 abortions were associated with pathologic ova.—*C.D.D.*

SMITH, O. W., G. V. S. SMITH AND D. HURWITZ. Increased excretion of pregnanediol in pregnancy from diethylstilbesterol with special reference to the prevention of late pregnancy accidents. *Am. J. Obst. and Gynec. 51* (3): 411–415 (1946).

A brief resume is presented of the hypothesis that the premature deficiency in the production of estrogen and progesterone characteristic of accidents of late pregnancy might be prevented by the oral administration of diethylstilbesterol. A diabetic woman with a poor obstetrical history was treated with diethylstilbesterol continuously, except for two short intervals, from the sixteenth through the thirty-fifth week, and weekly urinary pregnanediol levels were studied. The levels of pregnanediol fell precipitously whenever the treatment was interrupted and the pregnancy went to term uneventfully.

The authors propose a trial of oral diethylstilbesterol as a preventive measure in patients with a history of repeated accidents of pregnancy which may be referable to progesterone deficiency, namely, abortion, premature delivery, pre-eclampsia, eclampsia, or intrauterine death.—*C.D.D.*

VIERGIVER, E. AND W. T. POMMERENKE. Cyclic variations in the viscosity of cervical mucus and its correlation with amount of secretion and basal temperatures. *Am. J. Obst. and Gynec. 51* (2): 192–200 (1946).

Daily observations of basal body temperature and the viscosity of the cervical mucus were made on four normal young women. One was followed through two cycles and the others through four. The term viscosity is defined as the time in seconds required to draw a column of mucus through the capillary tube a given distance at a given pressure, as measured by a mercury manometer. In two of these cycles, the correlation between viscosity and penetrability was also studied. The method of Lamar, Shettles and Delfs was used in determining the penetrability. The rate of migration of the spermatozoa through a capillary tube filled with cervical mucus was estimated by means of a microscope, stop watch and calibrated mechanical stage. These studies appear to indicate that in normal subjects the ovulatory process is characterized by a period of increase in secretion and a decrease in the viscosity of the cervical mucus, along with a shift in the basal temperature.—*C.D.D.*

WINSON, S. G. An analysis of 257 cases of sterility. *Am. J. Obst. & Gynec. 52* (4): 631–634 (1946).

Of 257 patients studied, 53 had secondary sterility, and 204 primary sterility. A cervical factor was found in 38 per cent and following local treatment 75 per cent of these conceived. Ninety patients had partial and 50 total tubal occlusion. This was relieved and conception followed in 48 and 44 per cent of the patients respectively. Endocrine menstrual disturbances were found in 134 patients. A surprisingly large number of these, 82 or 31.9 per cent of the entire group, had anovulatory menstruation and 50 percent of these patients conceived. After proper treatment conception took place in 50 per cent of the 257 cases studied. There were 7 abortions and 1 ectopic pregnancy after treatment with equine gonadotropin or chorionic gonadotropin plus "pituitary synergist." The male factor was considered responsible in 40 per cent.—*C.D.D.*

WINTHER, N. Menorrheal problems in college women. *Am. J. Obst. & Gynec. 52* (5): 803–809 (1946).

In a 10 year study, 5,210 women were seen in the gynecological department of the University of Minnesota Student Health Service because of pain, vaginal discharge and irregular uterine bleeding. Thirty-two per cent of these presented themselves because of irregular bleeding. A brief resume of various types of treatment for menstrual irregularities is given.—*C.D.D.*